

1957
1958


Y
E
A
R

B
O
O
K









Digitized by the Internet Archive
in 2012 with funding from
LYRASIS Members and Sloan Foundation



YEAR BOOK 57

July 1, 1957—June 30, 1958

CARNEGIE INSTITUTION OF WASHINGTON
WASHINGTON, D. C.

1958

Library of Congress Catalog Card No. 3-16716
THE LORD BALTIMORE PRESS, INC., BALTIMORE, MARYLAND

CONTENTS

	<i>page</i>
OFFICERS AND STAFF	v
REPORT OF THE PRESIDENT	1
REPORTS OF DEPARTMENTS AND SPECIAL STUDIES	47
Mount Wilson and Palomar Observatories	49
Joint Committee on Image Tubes for Telescopes	89
Department of Terrestrial Magnetism	93
Geophysical Laboratory	167
Department of Plant Biology	261
Department of Embryology	307
Department of Genetics	373
Department of Archaeology	433
BIBLIOGRAPHY	457
ADMINISTRATIVE REPORTS	459
Report of the Executive Committee	461
Report of Auditors	463
Abstract of Minutes of the Sixtieth Meeting of the Board of Trustees	477
ARTICLES OF INCORPORATION	479
BY-LAWS OF THE INSTITUTION	483
INDEX	489

PRESIDENT and TRUSTEES

PRESIDENT

Caryl P. Haskins

BOARD OF TRUSTEES

Walter S. Gifford, *Chairman*

Barklie McKee Henry, *Vice-Chairman*

Robert Woods Bliss, *Secretary*

James F. Bell
Robert Woods Bliss
Lindsay Bradford *
Omar N. Bradley
Vannevar Bush
Walter S. Gifford
Crawford H. Greenewalt
Caryl P. Haskins
Barklie McKee Henry
Ernest O. Lawrence ‡
Alfred L. Loomis
Robert A. Lovett
Keith S. McHugh
Margaret Carnegie Miller
Henry S. Morgan
Seeley G. Mudd
William I. Myers
Henning W. Prentis, Jr.
Elihu Root, Jr.
Henry R. Shepley
Charles P. Taft
Juan T. Trippe
James N. White
Robert E. Wilson

* Resigned 1958.

‡ Died August 27, 1958.

TRUSTEES *Continued*

EXECUTIVE COMMITTEE

Barklie McKee Henry, *Chairman*
Robert Woods Bliss
Walter S. Gifford
Caryl P. Haskins

Robert A. Lovett
Henry S. Morgan
Henning W. Prentis, Jr.
Henry R. Shepley

FINANCE COMMITTEE

James N. White, *Chairman*
Walter S. Gifford
Alfred L. Loomis
Henry S. Morgan
Henning W. Prentis, Jr.

NOMINATING COMMITTEE

Elihu Root, Jr., *Chairman*
Robert Woods Bliss
Walter S. Gifford
William I. Myers

AUDITING COMMITTEE

Keith S. McHugh, *Chairman*
Alfred L. Loomis
Juan T. Trippe

RETIREMENT COMMITTEE

Barklie McKee Henry
Henry S. Morgan

COMMITTEE ON ASTRONOMY

Seeley G. Mudd, *Chairman*
Crawford H. Greenewalt
Elihu Root, Jr.

COMMITTEE ON BIOLOGICAL SCIENCES

Alfred L. Loomis, *Chairman*
Margaret Carnegie Miller
William I. Myers
Charles P. Taft

COMMITTEE ON TERRESTRIAL SCIENCES

Ernest O. Lawrence, *Chairman*
Barklie McKee Henry
Henning W. Prentis, Jr.
Robert E. Wilson

COMMITTEE ON ARCHAEOLOGY

Henry R. Shepley, *Chairman*
James F. Bell
Robert Woods Bliss
Juan T. Trippe

Note: Membership of Committees as of June 30, 1958.

FORMER PRESIDENTS and TRUSTEES

PRESIDENTS

Daniel Coit Gilman, 1902–1904 Robert Simpson Woodward, 1904–1920
 John Campbell Merriam, *President* 1921–1938; *President Emeritus* 1939–1945
 Vannevar Bush, 1939–1955

TRUSTEES

Alexander Agassiz	1904–05	Wayne MacVeagh	1902–07
George J. Baldwin	1925–27	Andrew W. Mellon	1924–37
Thomas Barbour	1934–46	Roswell Miller	1933–55
John S. Billings	1902–13	Darius O. Mills	1902–09
Robert S. Brookings	1910–29	S. Weir Mitchell	1902–14
John L. Cadwalader	1903–14	Andrew J. Montague	1907–35
William W. Campbell	1929–38	William W. Morrow	1902–29
John J. Carty	1916–32	William Church Osborn	1927–34
Whitefoord R. Cole	1925–34	James Parmelee	1917–31
Frederic A. Delano	1927–49	Wm. Barclay Parsons	1907–32
Cleveland H. Dodge	1903–23	Stewart Paton	1916–42
William E. Dodge	1902–03	George W. Pepper	1914–19
Charles P. Fenner	1914–24	John J. Pershing	1930–43
Homer L. Ferguson	1927–52	Henry S. Pritchett	1906–36
Simon Flexner	1910–14	Gordon S. Rentschler	1946–48
W. Cameron Forbes	1920–55	David Rockefeller	1952–56
James Forrestal	1948–49	Elihu Root	1902–37
William N. Frew	1902–15	Julius Rosenwald	1929–31
Lyman J. Gage	1902–12	Martin A. Ryerson	1908–28
Cass Gilbert	1924–34	Theobald Smith	1914–34
Frederick H. Gillett	1924–35	John C. Spooner	1902–07
Daniel C. Gilman	1902–08	William Benson Storey	1924–39
John Hay	1902–05	Richard P. Strong	1934–48
Myron T. Herrick	1915–29	William H. Taft	1906–15
Abram S. Hewitt	1902–03	William S. Thayer	1929–32
Henry L. Higginson	1902–19	James W. Wadsworth	1932–52
Ethan A. Hitchcock	1902–09	Charles D. Walcott	1902–27
Henry Hitchcock	1902–02	Frederic C. Walcott	1931–48
Herbert Hoover	1920–49	Henry P. Walcott	1910–24
William Wirt Howe	1903–09	Lewis H. Weed	1935–52
Charles L. Hutchinson	1902–04	William H. Welch	1906–34
Walter A. Jessup	1938–44	Andrew D. White	1902–03
Frank B. Jewett	1933–49	Edward D. White	1902–03
Samuel P. Langley	1904–06	Henry White	1913–27
Charles A. Lindbergh	1934–39	George W. Wickersham	1909–36
William Lindsay	1902–09	Robert S. Woodward	1905–24
Henry Cabot Lodge	1914–24	Carroll D. Wright	1902–08
Seth Low	1902–16		

Under the original charter, from the date of organization until April 28, 1904, the following were ex officio members of the Board of Trustees: the President of the United States, the President of the Senate, the Speaker of the House of Representatives, the Secretary of the Smithsonian Institution, and the President of the National Academy of Sciences.

STAFF

MOUNT WILSON AND PALOMAR OBSERVATORIES

813 Santa Barbara Street, Pasadena 4, California

Ira S. Bowen, *Director*; Horace W. Babcock, *Assistant Director*

Halton C. Arp	Jesse L. Greenstein	Donald E. Osterbrock §
Walter Baade *	Fred Hoyle	Robert S. Richardson §
William A. Baum	Rudolph L. Minkowski	Allan R. Sandage
Arthur D. Code ‡	Guido Münch	Olin C. Wilson
Armin J. Deutsch	J. Beverly Oke	Fritz Zwicky

DEPARTMENT OF TERRESTRIAL MAGNETISM

5241 Broad Branch Road, N. W., Washington 15, D. C.

Merle A. Tuve, *Director*

L. Thomas Aldrich	John W. Firor	Richard B. Roberts
Ellis T. Bolton	Scott E. Forbush	Howard E. Tatel ¶
Roy J. Britten	W. Kent Ford, Jr.	Georges M. Temmer
Bernard F. Burke	John W. Graham	Harry W. Wells
Dean B. Cowie	Norman P. Heydenburg	George W. Wetherill

GEOPHYSICAL LABORATORY

2801 Upton Street, N. W., Washington 8, D. C.

Philip H. Abelson, *Director*

Francis R. Boyd, Jr.	Gabrielle Donnay	Gunnar Kullerud
Felix Chayes	Joseph L. England	J. Frank Schairer
Sydney P. Clark, Jr.	Hans P. Eugster	George R. Tilton
Gordon L. Davis	Joseph W. Greig	Hatten S. Yoder, Jr.

* Retired June 30, 1958.

‡ Resigned August 31, 1958.

§ Resigned June 30, 1958.

|| Resigned February 8, 1958.

¶ Died November 15, 1957.

STAFF *Continued*

DEPARTMENT OF PLANT BIOLOGY

Stanford, California

C. Stacy French, *Director*

William M. Hiesey

Harold W. Milner

James H. C. Smith

Malcolm A. Nobs

DEPARTMENT OF EMBRYOLOGY

Wolfe and Madison Streets, Baltimore 5, Maryland

James D. Ebert, *Director*

David W. Bishop

Elizabeth M. Ramsey

Bent G. Böving

Mary E. Rawles

Robert K. Burns

Royal F. Ruth

Robert L. DeHaan

DEPARTMENT OF GENETICS

Cold Spring Harbor, Long Island, New York

Milislav Demerec, *Director*

Alfred D. Hershey

Margaret R. McDonald

Berwind P. Kaufmann

George Streisinger *

Barbara McClintock

DEPARTMENT OF ARCHAEOLOGY

10 Frisbie Place, Cambridge 38, Massachusetts

Harry E. D. Pollock, *Director*

Tatiana Proskouriakoff

A. Ledyard Smith

Anna O. Shepard

Robert E. Smith

Edwin M. Shook *

J. Eric S. Thompson

* On leave of absence.

OFFICE OF ADMINISTRATION

1530 P Street, N. W., Washington 5, D. C.

Caryl P. Haskins

President

Paul A. Scherer

Executive Officer

Samuel Callaway

Assistant to the President

Ailene J. Bauer

Director of Publications

Lucile B. Stryker

Editor

Earle B. Biesecker

Bursar; Secretary-Treasurer, Retirement Trust

James W. Boise

Assistant Bursar; Assistant Treasurer, Retirement Trust

James F. Sullivan

Assistant to the Bursar

Richard F. F. Nichols

Executive Secretary to the Finance Committee

STAFF *Continued*

RESEARCH ASSOCIATES

of Carnegie Institution of Washington

William A. Arnold, Oak Ridge National Laboratory

D. G. Catcheside, University of Birmingham

Hessel de Vries, University of Groningen

Louis B. Flexner, University of Pennsylvania

Willard F. Libby, University of Chicago

Paul W. Merrill, Mount Wilson Observatory

Jan Hendrick Oort, Leiden Observatory

Harry E. D. Pollock, Carnegie Institution of Washington

Hans Ramberg, University of Chicago

C. E. Tilley, Cambridge University

M. Westergaard, Universitetets Genetiske Institut, Copenhagen

Evelyn M. Witkin, State University of New York

R. v. d. Woolley, Royal Greenwich Observatory

CARNEGIE INSTITUTION OF WASHINGTON

REPORT of

THE PRESIDENT

CARNEGIE INSTITUTION OF WASHINGTON

REPORT OF THE PRESIDENT

The stage of romance is the stage of first apprehension. The subject-matter has the vividness of novelty; it holds within itself unexplored connexions with possibilities half-disclosed by glimpses and half-concealed by the wealth of material. In this stage knowledge is not dominated by systematic procedure. Such system as there is must be created piecemeal *ad hoc*. We are in the presence of immediate cognisance of fact, only intermittently subjecting fact to systematic dissection. . . . In our conception of education we tend to confine it to . . . the stage of precision. But we cannot so limit our task without misconceiving the whole problem. We are concerned alike with the ferment, with the acquirement of precision, and with the subsequent fruition.—Alfred North Whitehead in *The Rhythm of Education*.

The greatest of man's advances are made, not in the harshness of old necessity, but in the richness of new opportunity. With mankind, as in all life, grinding hardship to the limit of endurance may be met by sinewy resistance, may lead to extraordinary heightening of old skills, to a marvelous sharpening and extension of well tried modes of existence. But, in the larger sense, it may evoke little that is really new. Innumerable generations of some plant or animal species may live successfully and die peacefully in a stable and long-occupied environment and in the end show little evolutionary change beyond a host of minor adaptations, albeit exquisitely specific and precise. But just as the opening of some untried ecological realm to that plant or animal is characteristically met by a burst of large-scale evolutionary change, swift and often comprehensive, so it is against the great and novel challenge, in fresh and unknown gardens of the intellect and spirit, that the mind of man has always found unrecognized powers, has always gleaned strength and courage and capacity to reach new worlds.

On our well worn and crowded planet, the time has long since passed when new opportunities of this order can be grasped unless not only their exploitation but their very making are in large part the work of human hand and mind. In our world, the whole exploratory process has become essentially self-stimulating and self-creative, the spirit soaring on wings which, by necessity, are largely of its own fashioning. So every innovation of significance brings new opportunity in its train, and that new opportunity, in its turn, breeds innovation still wider and more encompassing.

It is clear that this situation, in essence, has been characteristic of the history of man at least since the tremendous discovery that crops could be reaped from the sown seed. But by its very nature the cycle from discovery to opportunity to new discovery is self-accelerating, in its later phases reaching explosive dimensions. So we could perhaps have predicted what is nonetheless a continuing miracle—the golden quality of our age, the incomparable richness of innovational opportunity in our time.

No vision of the scale of the heavens and earth's place within them of a Galileo or a Kepler or a Tycho Brahe, no revelation with the crude microscope of a Leeuwenhoek of a whole living world in microcosm which for an earlier generation was simply nonexistent, can have enlarged man's view of the universe more radically than some of the modern scientific advances of the last few years. That change of scale in knowledge and in viewpoint, so great in magnitude as in effect to differ in kind, is only partly represented by the new conquests of the physical universe by which today we often characterize it—the building of transuranic elements, the vast and varied potentialities inherent in nuclear fission and fusion, the advent of man-made satellites. It is only partly represented by the tremendous advances that mark our time in knowledge of the nature and evolution of the universe, of the structure of matter, of the quality of that immediate shell of space that lies hundreds or thousands of miles beyond our earth. It is only partly represented by the rate of growth of the scientific effort itself, which in England—and we can probably apply the relationship to the recent history of our own country without serious error—has been shown almost certainly to have doubled two or three times in a generation ever since the days of Newton. It is only partly represented by the present magnitude of the scientific and technical effort, merely suggested in our own nation in an estimate recently made by the Department of Economics of McGraw-Hill Publishing Company that in 1958 the expenditure of American industry alone for science and technology reached eight billion dollars. These are massive and powerful factors shaping and scaling our future opportunities for innovation. Yet even they may not be ultimately the most significant of our time.

The major revolutions in human thought—the silent revolutions of deepest import, when the basic nature of man's concept of the universe, of his relation to it and his purpose and goal within it, undergoes the greatest changes—are not only difficult to document long after they have occurred. They are largely invisible to those who pass through them, who have the high fortune to be their contemporaries. Even the great tangible events that may accompany such changes—the scientific conquests, the social upheavals, the political unrest and change—appear at the time largely as a series of striking but disconnected and disrupting events. In the perspective of three hundred years it is not very hard to see how interrelated were the turbulent material changes of the sixteenth and seventeenth centuries with the deeper revolutions of concept which accompanied them, or to realize how often that inner revolution was reflected in the more material, more evident, external one. Perhaps we too are privileged to live in a time of remolding of basic concepts that will ultimately be as fraught with opportunities for new exploration as was the scientific revolution of the sixteenth century—that may, indeed, form the most powerful of all the stimuli for innovation provided by our age.

Consider, for instance, the great changes upon which we may just now be embarking in our ideas of the very business of science, defined as the search for truth—the revolution which may come in our notions of the nature of scientific truth itself. When science is in its earliest observational stages of development, truth may mean largely the quantified, accurate description of phenomena, so made that any investigator who repeats the same observation under the same conditions will emerge with the same set of sensory impressions, and will be likely to draw similar conclusions from them. Thus it is perhaps fair to say that the earliest and most basic scientific “truth” was the sensory impression, verifiable by many men, of some feature of that world of phenomena which was conceived to lie outside of, away from, and unaffected by the observer. It was, perhaps, “knowledge” as Locke and his contemporary empiricists would have used the term.

This stage had already been outgrown by seventeenth-century science. Already the notions of truth and falsity had been extended beyond observations alone to the theories constructed to contain them. A prime criterion of merit was that a structure of cause and effect should be objectively defined, should be, as Isaac Newton emphasized, kept “free of occult influences.” That concept of cause and effect, of course, derived its immense influence in large part from the spectacular success of the new ideas of gravitation in providing theoretically calculated orbits to replace the descriptive treatment of the motions of the planets which had been current since the days of the Medievalists and their brilliant Arab co-workers—accurately predicting orbits which could be experimentally verified. Through all the following years to our own century, to the very beginning of the great revolution of thought upon which we are now well embarked, the accepted basis of scientific truth which experiment should test was, in large measure, whether other realms of matter would conform to the same laws. Such realms need not be perceptible to the senses. Much of the work of the later Newtonian physics, of course, was concerned with confirming the validity of those concepts of causality and truth in terms of molecular phenomena, in the theories of gases and of heat. It was here indeed that theory achieved some of its greatest successes, and experiment some of its major triumphs of technique and ingenuity. Through the greater part of three centuries, science advanced principally through a succession of physical observations, the building of hypotheses to connect them, the elaborating of results predicted from those hypotheses, the experimental testing of these results. These processes are still the essential building stones of scientific inquiry.

But in our own immediate day the revolutionary developments in nuclear physics and in relativity have made it clear that these straightforward procedures and the older logic that has so long connected them cannot, unaided, comprehend the whole of nature, or be adequate to all the needs of science. A door has been opened on vistas so wide and unfamiliar that the question seri-

ously arises as to how far a revolutionary science of the future can be, in the older conventional sense, strictly empirical or rational. Quite possibly the ultimate task of science, which we conceive in the context of our day simply as the search for truth, must be redefined in more comprehensive ways, not yet clear. Perhaps, following a suggestion of Martin Johnson compelling most sober reflection, we will come to think of scientific truth primarily as a measure of the communicability and coherence of systems of thought, as a property of interchangeability between observers on the one hand, and of scientific propositions between differing situations on the other. If the old definition of the primary task of science as the search for truth is to retain its essential meaning, we could then well say that the highest aim of scientific inquiry is to make knowledge communicable to all possible situations, and that knowledge shall be judged as "true" by the degree of "coherence" among such situations. It is particularly noteworthy that an essential core of such thinking, the concept of communication and communicability, may well be destined to become one of the central ideas of science in the years to come, coloring all its concerns, from particle physics to the sciences of life and mind.

It may be long before we achieve new ground with the certitude with which for three centuries we have occupied the old. But as we struggle to do so, opportunities—and indeed a fierce demand—for wide vision and penetrating originality will press upon us to a degree perhaps unexcelled in our experience. It is quite conceivable that when we have successfully met these new challenges, and embraced the opportunities that they will surely bring, we shall have completed an intellectual revolution no less profound than that which fashioned the modern from the medieval mind. Surely there has never been a time more rich in scientific opportunity.

At first glance, it seems curious that in an age which thus offers unparalleled challenges to break new ground, in an age which probably presents the richest fabric of novel enterprise in all human history, truly great innovation should still be a comparatively rare event. Even more striking is the rare and precious quality of the innovator himself, relative to the comparatively huge and ever-growing number of fine minds devoted to the scientific way. It is hard to escape the impression that the opportunities for original advance have not been as fully grasped as they well might be.

This rarity of innovation, and innovators, of course, is as old as man's experience. We are so accustomed to the notion of continuous progress in our modern world that we find it hard to comprehend that real progress has been the shining event, not the rule, of human history. The astonishing uniformity of some primitive artifacts used by early peoples over wide regions of the globe suggests how far each single original improvement of technique may have diffused by copying, and how influential it may have been in the advance of

all mankind. There is suggestive evidence that many elements which formed the material basis of the early civilizations of the Old World—and so provided the very foundation upon which our own culture was erected—trace their origins from a limited region of the Middle East. How wide the influence of a single cultural innovation, once made, can be, and how rapidly it can make itself felt, are vividly suggested in historic times by the speed of the spread of the culture of the horse among the plains Indians of the Southwest, or by the even more explosive propagation of the culture and use of the domestic fowl along the basin of the Amazon. It is strikingly attested in our own day in the rapidity and facility with which technological, as opposed to cultural and conceptual, innovations may be accepted and take root and flourish among societies otherwise of the most divergent background.

One reason for the historical rarity of great conceptual innovation in more recent times undoubtedly lies in the difficulty of its communication—not so much in its failure to be recognized as in the misconstruction of its message. The followers of Darwin, after his death, introduced all sorts of implications extraneous to his theory. They applied it to areas where it was quite inappropriate, which he was especially careful to avoid, generating misconceptions that have required two generations of intensive work to clear away. The discoveries of Gregor Mendel, the monk of Altbrunn, whose epoch-making experiments in the heredity of garden peas and the conclusions he drew from them were models of precision in measurement and clarity of reasoning, set the whole stage for modern genetics. But they were of necessity published in a local journal, obscure and little read, and so were lost for almost thirty-five years. When they were finally unearthed in 1900, Mendel was already dead, and those who found his paper rediscovered only a part of his conception. They saw in his work, not his vision of a wide and grand design, but rather much narrower and more specific findings, to be tested by their own kinds of experiments. For this failure of communication, the whole science of genetics suffered for many years.

The intrinsic difficulty of great innovation and the numerous impediments to its communication are surely but two of the reasons for its comparative infrequency. An important and perhaps an ultimately limiting factor, of course, is the inherent rarity of the deeply innovating mind. Perhaps we must accept as given the fact that in any human population there will be a low proportion of truly original figures. But we are far from certain just how low that proportion needs to be under the best conditions. The nature of those conditions demands the most serious consideration.

It is a striking paradox that, though our contemporary culture affords opportunities for creativeness probably unequaled in human history, it also poses hazards that may likewise be unequaled. One important kind of hazard is inherent in the very structure of thought and philosophy which may crystallize

about a field of inquiry. Such well formed systems, when rigidly and tenaciously held, may constitute more powerful and dangerous barriers to the advance of concept and experiment alike than we ordinarily recognize. The more elaborate the pattern, the more adequate, the more satisfactory, the more self-consistent it seems, the more difficult it is to transcend and the greater may be the resistance to truly original departures from it. This paradoxical balance between the older thinking and the new is the more delicate because it seems inevitable that, although all new scientific concepts must transcend the older matrix from which they arise, if they are to become established and permanent they must also find firm links with it.

This dual relationship between the new advance and the older structure of knowledge and reasoning from which it springs may be significant in shaping the history of human thought so characteristically to long interludes of refinement and consolidation, punctuated by shorter periods of explosive revolution. The record is replete with examples of the power and persistence of a great central idea and the elaborate ways in which new and potentially revolutionary thought, for better or worse, may long be organized around it or accommodated within it. Through a whole age, truth in observation for the medieval mind was measured by the success with which impressions of the world could be selected and organized about current religious images. What we would call symbolic meaning was for that age, in large measure, its fundamental reality. Research in its modern sense was retarded for at least a hundred years by the belief, still powerful in the days of colonial New England, that all knowledge about the world is given in advance of an investigation, is hidden within it like the imaginary statue in a block of marble, awaiting only the master's chisel; that the most that right reasoning can possibly do is to make it stand forth more clearly and brilliantly revealed; that there is therefore nothing essentially creative in the whole process of investigation of nature. It required a major revolution to reach the view that the investigation itself might determine the concepts which would follow it, and that reality might be more closely oriented toward the primary evidence of our senses. It was a revolution vividly symbolized, as Lewis Mumford has pointed out, by the general introduction into men's homes of clear glass windows letting in a wide and literal view to replace the great stained glasses of the medieval world, with their brilliant slanting shafts of colored light, mirroring symbolic images already fixed and finally conceived.

The revolution that replaced the medieval by the essentially modern outlook was comparatively swift and violent, once the old containing vessels had been broken. But the wonder, perhaps, is not so much in their fragility as in their elastic and confining durability. Other, similar vessels of concept, fashioned at almost as remote a time, only now are beginning to show the fine cracks that in our own generation may presage another revolution as great.

There can be no more vivid examples of the tremendous power of a central

idea in advancing or retarding innovation than those offered by the sciences of life and of mind, in our generation in ferment such as they have not known since the days of Galen or of Leeuwenhoek. The distinction between the "living" and the "not-alive" as two quite different and opposite categories is far older than even medieval science. From the days of Harvey it was refined even further, until, for almost-modern biology, it was not considered difficult in practice to characterize a newly discovered object as living or nonliving. Into our own time the life sciences almost unconsciously retained as a central concept a notion that actually had its greatest currency in sixteenth-century thinking. Implicitly, if not explicitly, the organism was commonly endowed with the "property" of life as a unique, inhabiting quality much as the alchemist endowed the burning body with phlogiston, or the early physicist the bar with weight and color, length and hardness, or the physician the bitter pill with taste.

Perhaps what did as much as anything to shake the central idea of life as a unique "inhabiting" property was the preparation of the "crystallizable" plant viruses a quarter of a century ago and the discovery of their long durability upon the shelf, so like a "lifeless" chemical, combined with the power to resume the lifelike properties of growth and reproduction after long periods when re-inoculated to a suitable host. With that step taken, with the recognition that the "living" and the "nonliving" may in some contexts at least differ in degree rather than in kind, the advances of concept and experiment that now are in full tide crowded upon one another. Recent studies of the dynamics of metabolism, especially in microorganisms, have helped to replace the older notions of the structures of life as gross and essentially static morphologies, like cell walls and semipermeable membranes, with those far more exciting concepts of precise structural lattices and highly specific sites of adsorption which in the last years have become dominant. Radical experiments in the synthesis of amino acids under conditions approximating those believed to have obtained on the lifeless earth, and illuminating researches in paleobiochemistry, have made common currency of the notion that living things may have indeed originated terrestrially, and have further obscured the division between the living and the nonliving. Quantitative biochemical genetics, the fine analysis of the structure and function of the chromosome that is proceeding apace today, with its accompanying new insights into the mechanisms of replication and its demonstrations of transduction and transformation, further reinforce the new ideas of biological systems as wonderfully complex dynamic structures of exquisitely detailed precision in both space and time. Some modern investigators of the structure of the chromosome see in it a precisely designed mechanism for the transfer of information, as truly an instrument of communication as any designed by man, and far more intricate in function. Indeed, it seems quite possible that in our time some of the new central ideas about living matter, whether as

individual organism, as structured population, or as the changing product of evolution, will describe it primarily in terms of self-regulating, goal-directed systems to which modern concepts of information theory and information flow can profitably be applied. It is hard to realize the swift and tremendous strides of thirty years which the escape from too exclusive a preoccupation with an old idea has brought.

A similar revolution is beginning in the science of the mind, again consequent upon the abandoning of an older concept, this time even more confining. Here, to the notion of life as a "property" of living matter was added a further barrier—the central idea of the mind as fundamentally different in kind even from the living body, the *res cogitans* as distinct from the *res extensa*—inherited from the brilliant eighteenth-century advocacy of Descartes. It is only in the last few years that mind, too, has widely come to be regarded as a self-regulated, goal-directed dynamic system, structured, like life itself, with exquisite precision and almost unimaginable complexity in both space and time, but not necessarily differing in essence, not necessarily wholly beyond the modes of investigation already available to us. It is only now that the central idea of communication which has become so prominent in the life sciences—and indeed may be so important to the whole structure of science itself—has been extended to the subject of mind as well; that mental processes in some of their aspects are beginning to be translated, like the processes of heredity, into terms of communication theory and information flow. We can only speculate today what the consequences may be. As with the life sciences, experimental evidence is accumulating along the novel paths of thought that escape from the older pivotal notions has made possible, evidence provided from such diverse sources as studies of therapeutic neurochemicals and of electronic computing systems.

No more vivid historical examples, perhaps, could be cited of the long delays to basically new departures that too static and finished, too self-contained and self-consistent systems of thought may impose than these sciences of life and mind.

There are other barriers of a more practical sort. One of the profound ways in which the modern world may differ from other centuries is in its conscious recognition of, and its high respect for, the *fact*—but not always the *process*—of innovation. Recognizing its power, our own society would like to hurry it along, to enlarge its scale, to organize it more efficiently. Impressed as we are by the extent to which the processes of development built upon innovation can be accelerated by organization, we are greatly tempted to extend this thinking to creative matters, to believe that we can organize for great innovation too. Such misconceptions are peculiarly liable to occur in massive and highly organized environments devoted to research and development. They are inherent in the all-too-prevalent confusion between the processes of scientific

research itself and of development, in the frequently uncritical transfer of ideas and techniques from development and production into the field of scientific innovation. Among them, too often, is the destructive concept that the effectiveness of innovation, like that of production, can be doubled simply by doubling the *size* of the accompanying effort. Some departments of research and development in industry and in government are particularly vulnerable, and the academic world is by no means immune.

But we should be well warned. It is deeply significant that, in our own time of unparalleled technical advance, in a day when engineering achievements built about new principles can accomplish unbelievable things, the great innovations are still highly individual, are still associated, at their inception, with a mere handful of names.

The comparative rarity of the environments most favorable to truly original investigation, their unusual and special character, and the frequent practical impediments to their best development may impose sharper limitations on the creative potential of our nation than we are inclined to recognize. Climates of research must comprehend many and varied elements, some of which are rarely joined in more ordinary situations. Important among them are understanding and protection. Since innovation is and must always remain a uniquely individual experience, it poses unusual social dangers for the investigator from which he must be adequately shielded—the animosity which the new, with its threat to current things, must often provoke. Such perils may not be fatal to the advance once it is fairly made. For if the advance is truly novel and important, and if the society in which it occurs is self-confident, it is likely to be eventually accepted and incorporated, and may even become symbolic. The greater risk is that the prospect of such hardships may inhibit the individual capable of great innovation from undertaking it or from fully developing its results. Such considerations of the hazards opposing the unexampled richness of opportunity for scientific creativeness in our society bring home anew the gravity of our responsibility to analyze most carefully this climate in which true scientific originality best flourishes and to see that environments embodying it are adequately provided and conserved.

It is not hard to set down the major tasks that institutions assuming these great responsibilities must perform. The most important, of course, must be the discovery, the encouragement, the adequate training of the outstandingly original investigator, and the fostering of his work. Such institutions must be unusually sensitive to the rare, natively innovating mind, and unusually adept in its discovery. They must have in high degree the ability to promote and develop the peculiar drive, the special verve and flare, the fine sense of style, which great innovation inherently requires. They must be able to train the

innovator of exceptional promise in the company of other gifted investigators already mature and at the height of their powers.

Above all, the environments that such institutions create must be unconventional and malleable. Their responsibility is not only to provide for the very different requirements of different workers, based in divergences of temperament, of method, of timing. It is also to meet the very different requirements of the same investigator at various periods of his working life. There must be ready, yet penetrating, astringent tolerance—and indeed welcome and encouragement—for the long preliminary phase of apparently rambling and aimless effort that so often and so characteristically precedes the great individual discovery or the brilliant innovational career, while the ground is being canvassed and a wealth of divergent but relevant material is being mastered and consolidated. There must be provision for the long intervals of seclusion and introversion that may be essential during periods of highest creativity, when white-hot effort will not brook interruption. There must be ample understanding and ample capacity to shield the worker in this his most vulnerable time from the insistent demands that will surely come to prematurely formulate results and conclusions only half-attained. Conversely, there must also be adequate provision for compensating periods of extroversion, when the immediate barrier is surmounted and the conclusions are consolidated and the extensive communication that must accompany or follow creativity is encouraged and sustained.

Institutions for creative research must be able to set and apply subtle standards of excellence. They must be able to judge and reward high standards by criteria more searching and more true than those too often and too superficially accepted. They must provide teachers of research of the very highest quality, and in high ratio to those they train—indeed, those primarily in training as investigators should, ideally, be in the minority. And they have one final and supremely important responsibility—the task of communicating the results of research rapidly and effectively to workers in fields that may seem far remote, yet may actually be contiguous to those in which the discoveries have been made. Such institutions will benefit greatly by being relatively small, and will almost certainly benefit, too, if they are somewhat decentralized, both physically and in the subject matter with which they deal.

The discovery and training of new generations of investigators in science are, of course, basically functions of the universities. Theirs must be a grave portion of the responsibility. But they are inevitably hindered in this special task by many of the current pressures that bear especially heavily upon them: the pressures to large size, to an ever-lessening ratio of teachers to students, to a lowered quality in the teaching staff itself, valiantly as this is—and must always be—resisted. Moreover, the whole mission of the universities is of course far broader. The training of great innovators, and, even more specifically, of great

innovators in the scientific way, must always remain one of their most important challenges. But it is, of necessity, a somewhat narrow sector in the total context of their work. They need assistance.

Such circumstances place renewed emphasis in our day on the great and continuing importance of the research institute in our national life. Its task is not only that of scientific innovation and discovery. This indeed is important enough. But, far more important, to it must fall the essential function of symbolizing in the best and purest form the way of scientific innovation with all that it means for our nation and our culture—undismayed and undeterred by pressure or by hazard. To it belongs the great responsibility of discovering and fostering new minds of high promise, of selecting them with discernment, of affording them a rare training as investigators, and then of seeing to it that their careers can be fulfilled where their talents are most effective. Far beyond the task of research itself lie the challenge and the responsibility to cultivate new minds through all the possible and relevant means—symposia and exchange fellowships, the flow to university and industry and back again, all the particular media of publication.

More and more, in our time, it is recognized that the true wealth of a nation resides not so much in the volume and the variety of goods that it can produce, not even so much in its total resources of production, but rather in two human factors more subtle, more powerful, more determining: in the quality and excellence of its people as a whole, and in their capacity for innovation. In the guarding, the fostering, the building of this last element of national wealth lies the ultimate challenge to such a research organization as the Carnegie Institution of Washington. Never in the history of science in our nation has that challenge been more exciting, that responsibility more keenly privileged.

THE YEAR'S WORK IN REVIEW

The past year has been an unusually full and active one in many facets of the work of the Carnegie Institution. It has been notable for the number and variety of investigations going forward, both those newly initiated and those continuing from other years. In consequence, it is even more difficult than usual to select individual projects for this review on other than a largely arbitrary basis. The ones described are intended only as representative examples of the kind of work in progress through the year. They may not be more or less important, more or less striking, than others that might equally well have been included. The reader interested in following the programs of the various departments in greater detail should turn to their individual and more complete accounts, which follow.

This year is noteworthy in other respects. It marks the tenth anniversary of the dedication of the 200-inch Hale telescope on Palomar Mountain and the tenth anniversary of the agreement for the joint operation of the Mount Wilson and Palomar Observatories by the Carnegie Institution of Washington and the California Institute of Technology. It also marks the termination of the work of the Department of Archaeology (though not of all the work of the Institution in that field), bringing to a close a record of distinguished pioneering achievement in the history of the Maya in Middle America. It has been a chapter of exploration not only extremely productive in its own right, but one that largely shaped the standards and set the stage for all the following investigations in that field. It seems particularly appropriate in this report, therefore, to present a brief review of the past ten years of the Mount Wilson and Palomar Observatories, and of the entire program of the Department of Archaeology.

Ten Years of Mount Wilson and Palomar Observatories

The ten years since the Hale telescope came into operation comprise an exceedingly notable decade in astronomy, marked by very important advances in knowledge. Two such advances demand special notice because of their general and fundamental character. The first relates to the changes that have come in our ideas of the size of the observable universe, the magnitudes of the distances within it, and its age. The second concerns the equally striking gains in our knowledge of the ways in which stellar evolution occurred in the past and is currently taking place.

Between the two world wars Edwin Hubble made an extensive series of measurements of the distances, diameters, and luminosities of the galaxies with the 100-inch telescope on Mount Wilson. His findings provided definite evi-

dence that the galaxies are huge systems of stars, similar to our Milky Way, and that they form the major units in which the mass of the universe is distributed. When the Hale telescope became available, a thorough restudy of the near-by galaxies was a major project. It was planned in particular that the procedures used in fixing galactic distances be re-examined, to reduce or eliminate the large uncertainties known to exist in the initial measurements. In general, such procedures involve the comparison of the apparent brightness of some specific distance indicator in the galaxy, such as a star, with its brightness or luminosity as determined from near-by examples taken from our own Galaxy. The required answer is obtained by applying the inverse square law for the falling off of apparent brightness with distance. To be useful as a distance indicator, the star or other object must be unusually bright and must have some special characteristic, to permit its identification as a particular type with the same brightness as examples from our own system. Cepheid variables were the classic distance indicators used by Hubble. They are particularly convenient because of the ready identification made possible by their light-fluctuations.

In 1952 the conclusion was reached that the classic cepheid variables are about 1.5 magnitudes brighter than had been thought from earlier work. Independent observations by Walter Baade with the Hale telescope, and by A. D. Thackeray and A. J. Wesselink on the Magellanic Clouds at Pretoria, together with theoretical studies by Henri Mineur and by Adriaan Blaauw and H. R. Morgan of proper motions of cepheids in our own Galaxy, all pointed in this direction. A careful remeasurement was therefore undertaken of the apparent magnitudes of a substantial number of cepheids in several of the nearer galaxies.

The absolute luminosity of a cepheid variable is a function of its period. Several dozen plates must therefore be made of each field of view, distributed over a sufficient time so that a reliable light-curve can be obtained of each variable. Sets of plates were obtained for this purpose by Baade for four fields in the Andromeda galaxy and for several other galaxies in the local group.

While this program of remeasurement of apparent magnitude was in progress, another approach to the same question was being explored simultaneously—the investigation of properties of other unusually bright objects that might be useful as distance indicators. Hubble and Allan R. Sandage measured the brightest stars in the Andromeda galaxy. Sandage investigated the integrated magnitude of the gaseous nebula or H II regions surrounding several of these very luminous stars. Arthur D. Code and T. E. Houck compared the exceptionally bright blue stars in NGC 6822, M 33, the Large Magellanic Cloud, and our own Galaxy. Halton C. Arp made a very extensive study of the magnitudes, light-curves, and frequency of occurrence of ordinary novae in the Andromeda galaxy, photographing fields in that galaxy with the 60-inch tele-

scope on every clear, moonless night for two observing seasons. Thirty novae were found during this period. Measurements of the distribution in magnitude of the globular clusters in the Andromeda galaxy were made by William A. Baum. Milton L. Humason and Sandage made observations of the red supergiants in M 33. Hubble, Sandage, and Baade observed various distance indicators in the more distant galaxies outside the local group.

Lastly, it was necessary to recalibrate the magnitudes of the stars in specific Selected Areas that had been used as standards of magnitude for the very faint stars in these galaxies. Such a recalibration, using photoelectric techniques, was started by Dr. Joel Stebbins and Dr. A. E. Whitford of the Washburn Observatory, using the 100-inch telescope; it was completed by Baum with the Hale instrument. It developed that the earlier recorded magnitudes of very faint stars, photographically determined, were too bright by as much as one magnitude.

When various corrections were made, Baade calculated in 1952 that the Andromeda galaxy is 2 million light-years distant—nearly three times as far away as the previously accepted value. By the same token, its diameter and mass must be increased by a similar factor, and its luminosity by a factor of about 8. The application of the new values of the magnitudes of various distance indicators to the preliminary observations of more distant galaxies points to an even larger correction to the distances and dimensions of these objects. Thus Sandage, in a preliminary study, during the current year, of the distances of the farthest galaxies in which individual distance indicators can be observed, estimates that the old distances of the Virgo cluster and all more remote galaxies must be increased by a factor of between 5 and 10 over the values obtained in the 1930's. If confirmed by further measurements, this leads to the striking conclusion that estimates of the diameter of the observable universe must also be increased by a factor of 5 to 10. Finally, if the length of time since the beginning of the expansion of the universe as indicated by the redshift is taken as the age of the universe, this age is increased by approximately the same factor, that is to between 7 and 13 billion years—a revision that brings the age into better agreement with other age determinations, such as that of the solar system.

During World War II Baade took advantage of the unusually good observing conditions afforded by the widespread black-out in the Los Angeles area to reinvestigate the types of stars in the nucleus and in the spiral arms of the Andromeda galaxy and in the elliptical galaxies, using the Mount Wilson telescope. He found that the most conspicuous stars in the galactic nuclei were red giants, while in the spiral arms of Andromeda and in the neighborhood of the sun in our own Galaxy the brightest stars were blue. From these observations the concept of differing population types of stars arose, the stars in our own neighborhood being designated as Population I and those in the galactic nuclei and in the elliptical galaxies as Population II.

An extensive study of the properties of the stars in the two populations was

instituted. Measurements of the color-magnitude relations were made for various groups of stars, including several globular clusters and galactic clusters, observed by Arp, Baum, Donald E. Osterbrock, Sandage, Maarten Schmidt, and Merle F. Walker, and including a few members of the local group of galaxies investigated by Baade and Sandage. Studies were also undertaken of the period-luminosity function of cepheid and cluster-type variables in these objects.

From these studies the picture soon emerged that Population I is characterized by very young stars, only recently condensed from clouds of dust and gas. Population II, on the other hand, contains only old stars. Parallel theoretical and laboratory developments, showing that the energy radiated by the stars comes from the transformation of hydrogen into helium and heavier elements in the hot stellar core, confirmed this picture. Detailed analyses of the nuclear processes occurring in these stars were developed by Fred Hoyle and G. R. Burbidge in collaboration with Dr. W. A. Fowler and Dr. E. M. Burbidge of the Kellogg Radiation Laboratory. The effect of the depletion of the hydrogen fuel on the characteristics of a star was investigated theoretically by Hoyle and by Dr. Martin Schwarzschild and others.

The picture of stellar evolution that emerges is, in general, as follows. When a mass of gas consisting predominantly of hydrogen condenses into a star on what is called the main sequence, its luminosity and surface temperature on this sequence are fixed by the mass condensed, the luminosity varying approximately as the cube of the mass, the surface temperature increasing slowly but continuously with the mass. Rapid expenditure of their hydrogen fuel by the very massive stars soon exhausts it. As the fuel becomes depleted, the star expands. Its surface cools while its total radiation is increased. At this critical stage the star leaves the main sequence. As the fuel is finally exhausted the radiation rapidly falls off and the star probably ends its career as a faint white dwarf.

On this picture, a young group of stars is characterized by a continuous progression of properties from massive, extremely luminous blue stars to the reddish faint dwarfs. As the group ages, the very luminous blue stars exhaust their fuel and drop out. The brightest stars in such a group are those of moderate initial luminosity which with approaching fuel depletion have expanded and cooled off, but have temporarily become more luminous. Such a stellar group, a few billion years old, should have the distribution in luminosity and color found in fact to exist among the globular clusters and the elliptical galaxies.

The discovery of the role played by nuclear transformations in the evolution and energy production of the stars gave an added interest to spectroscopic studies of the abundances of the elements participating in these transformations in stars of various ages. The great light-gathering power of the Hale telescope and the unique efficiency of its spectrographic equipment have made possible

the intensive study of faint stars, such as the globular-cluster stars and the white dwarfs, of special significance in evolutionary theory.

Extensive studies of the properties of the stars in globular and galactic clusters have been made during the current year. All the stars of a given cluster are at essentially the same distance and are believed to have about the same age. Determinations of the apparent magnitude and color of the stars in these clusters yield at once the data on the relationship between luminosity and surface temperature for groups of stars of various ages that are so essential for the interpretation of stellar evolution. A study of NGC 188 by Sandage and Dr. Sidney van den Bergh of the Perkins Observatory gave evidence that it is about 1.5 billion years older than any previously known galactic cluster. Thirty-two white dwarfs, which are supposed to represent the final stage in stellar evolution, were discovered by Baade in the globular cluster M 67. From spectroscopic studies, Jesse L. Greenstein, with the assistance of H. Lawrence Helfer and George Wallerstein, found that the abundance of the metals in the stars of the globular cluster M 92 is only 0.5 per cent of that in the stars in the solar neighborhood, whereas in the cluster M 13 it is about 5 per cent.

Armin J. Deutsch has found important evidence of a process by which the massive stars, when in the expanded red-giant stage, eject into space an appreciable fraction of their mass, providing a probable explanation for the great differences between the initial mass of the blue giant stars and their final mass in the white-dwarf stage, and explaining the presence of the large number of atoms of the heavy elements observed in the stellar gas.

A most important field of investigation was opened by Hubble in the third and fourth decades of the century with the discovery of the linear relationship between the radial velocities of the galaxies and their distances. In the 1920's and the 1930's these velocities were extensively measured with the 100-inch telescope by Humason. When the Hale telescope was completed, Humason used this instrument and its prime-focus spectrograph to measure the radial velocities of a substantial number of additional galaxies. In particular, he extended those measurements to very distant objects having a velocity of recession about 20 per cent that of light. At the same time Stebbins and Whitford of the Washburn Observatory and Edison Pettit of the Mount Wilson and Palomar Observatories used the 60-inch and the 100-inch instruments to measure photoelectrically the magnitudes of the galaxies whose velocities had been determined. In 1956 Humason, Sandage, and Dr. N. Mayall of the Lick Observatory published a list of the velocities of 600 galaxies measured at Mount Wilson and Palomar, together with 300 galaxies observed at the Lick Observatory. A detailed analysis was then made of the velocity-distance relationship, based on the observed velocities and the distances deduced from magnitudes.

A new method for the measurement of the redshifts of very faint and distant galaxies has been developed by Baum. Employing eight-color photometry,

using wavelength bands distributed uniformly throughout the spectrum, he has determined the shape of the curve relating radiated energy to wavelength for a number of galaxies at a wide range of distances. The displacement of this curve for a distant galaxy with respect to that of a near-by galaxy gives a measure of the redshift. Redshifts as large as $\Delta\lambda/\lambda=0.4$, nearly twice the largest definite redshift obtainable from spectroscopic observations, have been found. Indeed, it is becoming evident that the primary factor that sets the limit on the distance to which the Hale telescope—or any telescope of similar magnitude—can observe a galaxy or a cluster of galaxies is not the falling off of the light because of the increasing distance. Instead, it is the redshift of the light from the galaxy into the far infrared, where photographic plates are no longer sensitive, where atmospheric absorption is large and atmospheric “night-sky” is strong.

Observations have been made to investigate various properties of the galaxies. Thus Guido Münch has made spectroscopic observations of M 81 to determine its rotation and mass. Jan Hendrick Oort and Rudolph L. Minkowski have investigated the rotation, the mass distribution, and the range of stellar velocities in NGC 3115. Multicolor measurements of the integrated light from a large number of globular clusters and galaxies have been made by William G. Tifft for a study of their stellar contents. Similar studies of the energy distribution within galaxies have been made by Code, using a spectroscopic scanner. Fritz Zwicky has carried out studies of the luminosity function and the distribution and clustering of galaxies, and has also investigated the faint blue stars and other objects in the halo about our own and other galaxies.

Following the discovery of strong radio sources in space, Baade and Minkowski were able to identify several of the strongest of these with peculiar celestial objects which had already been observed optically. Several of these radio sources, such as Cygnus A, NGC 1275, and probably NGC 5128, have proved to be in fact pairs of galaxies in collision. It is thought that collision of the gas clouds in the spiral arms of these pairs of galaxies is responsible for the strong radio waves they emit. The remains of two supernovae which exploded earlier—the Crab Nebula, known to have exploded in A.D. 1054, and a faint nebula in Cassiopeia first observed by Minkowski and identified by him as the remains of the Supernova of 1572—have been shown to be identical with radio sources. The discovery in the Soviet Union of the polarization of the light from the Crab Nebula, followed by the detailed studies of this polarization by Baade, confirms the suggestion that much of the light observed from this object is synchrotron radiation from electrons of very high velocity accelerated in a small magnetic field. Similarly, the observations by Baade of the polarization of the light from the jet of the Galaxy M 87, also a radio source, points to synchrotron radiation as the source of much of its light.

The discovery in 1946 by H. W. Babcock of a star having a general magnetic

field with a strength of several thousand gauss opened a field of great interest. It has been followed by a survey of many similarly magnetic stars. In all, 305 stars have been examined from this point of view. Of these, 84 show a definite magnetic field. It is probable that there are such fields in 55 additional ones. Since in many stars the magnetic fields fluctuate more or less regularly with a period of a few days, it has been necessary to make extensive observations of many of these objects over a long period of time. The spectroscopic and other properties of these magnetic stars have been extensively studied by Deutsch in an attempt to construct a model to explain their behavior.

With the development of the solar magnetograph by H. D. and H. W. Babcock, it has become possible to map the magnetic fields over the surface of the sun with an accuracy of a few tenths of a gauss. Daily maps, taken over a period of a few years, provide definite evidence of persistent polar fields and of fluctuating fields over the equatorial regions of the sun. The observed fluctuations explain why the early attempts of Hale and of others to measure a general field were inconclusive. Sunspots, prominences, flares, and other solar phenomena have been investigated by Seth B. Nicholson and Robert S. Richardson on photographs and spectroheliograms taken daily at the solar towers on Mount Wilson.

Extensive studies have been made of the gas clouds within our own Galaxy. Surveys carried out by Minkowski have nearly doubled the number of known planetary nebulae. Spectroscopic techniques have been developed by Olin C. Wilson which have enabled him to study the internal motions of the planetary nebulae and, with Münch, to map the motions of the gases over a large part of the brighter areas of the nebula of Orion. Osterbrock has used a new technique to measure the densities of the gases in various nebulae.

During this period Ralph E. Wilson completed the radial-velocity program of the Observatories. This involved the measurement of several thousand plates, many of which were accumulated during World War II, and the publication of the radial velocities of more than 2400 stars. Under the title *General Catalogue of Stellar Radial Velocities*, Wilson has published a compilation of the positions, magnitudes, spectral types, and definitive radial velocities of all the 15,105 stars whose velocities have been determined at any observatory.

Olin C. Wilson, with the assistance of M. K. Vainu Bappu, has made a detailed study of the widths of the emission components at the center of the H and K lines of the spectra of a number of late-type stars. He discovered a linear relationship between the logarithm of this width and the absolute magnitude of the star which should prove a powerful tool in determining the distances of stars of these types. A large number of observations have been made by Paul W. Merrill, Alfred H. Joy, and Roscoe F. Sanford on the spectra of variable stars, and of stars with prominent emission lines.

A major program of mapping, including the whole sky north of declination -27° , carried forward under the sponsorship of the National Geographic Society, occupied most of the present decade. Two photographs were taken of each of 879 separate fields, one in blue light and the other in red light. Stars were recorded to the twenty-first magnitude on the blue plates and to the twentieth on the red plates, achieving a penetration to a distance about three times farther than any previous surveys—representing a coverage of about twenty-five times the volume of space. More than 120 copies of this National Geographic-Palomar Observatory Sky Survey, containing 1758 photographic prints, have been ordered and are being distributed to observatories throughout the world. From a search of the survey plates George O. Abell compiled a list of 2712 galaxies, of which but a few dozen had previously been known.

This year Sandage and E. M. Burbidge have found that two faint globular clusters discovered by Abell in the Survey lie at a distance of 400,000 light-years. As this is twice the distance of the Magellanic Clouds these clusters are clearly intergalactic.

Two very unusual asteroids, named Icarus and Geographos, which may come unusually close to the earth, were discovered just before and during the Survey from observations made with the 48-inch schmidt camera. Another discovery of a new object, comparatively near by, was made in 1951, when Nicholson detected the twelfth satellite of Jupiter on plates taken with the 100-inch telescope on Mount Wilson.

Shortly before the initiation of the joint operation of the Mount Wilson and Palomar Observatories, a guest-investigator program was inaugurated. Its purpose was to make the unique facilities of the Observatories available—to the extent that they were not required by the regular staff—to a wider group of astronomers from other institutions. This program has been carried forward consistently on a cooperative basis throughout the decade. The institution from which the visiting astronomer comes provides the necessary leave and travel expenses for him; the Observatories furnish the telescope time and the photographic plates and other supplies for the proposed program. During the decade, 60 astronomers from 23 institutions in the United States have made more than 160 visits to the Observatories to carry on work directly with the telescope, or in a very few cases to study plates already available in the files. In this same time 20 astronomers from 18 institutions in 12 other countries have made 26 visits.

The optical tests of the Hale telescope, which were reported early in the decade, have shown that the instrument performs as well as had been planned. But the real, functional test of any great new instrument, and of the thinking and planning that lie behind it, is the degree to which it can solve new problems, the extent to which it can break new ground. The record of the first

decade of operation of the 200-inch telescope and its supporting instruments on Palomar Mountain and Mount Wilson—the record of the past ten years of the Mount Wilson and Palomar Observatories, in short—provides the final and crowning evidence of the vision of George Ellery Hale, embodied in the great and unique instrument that bears his name.

The Department of Archaeology

This year marks a major occurrence in the program of the Carnegie Institution—the completion of the work of the Department of Archaeology. It signalizes the accomplishment of a most noteworthy—indeed a most outstanding—undertaking, which took its origin in the Institution more than fifty years ago, the records of which fill approximately a hundred volumes of Institution publications and include many papers published elsewhere, and which culminated in the extraordinary pioneering investigations of Middle American aboriginal culture for which it is best known.

It is clear that consideration was given to research in various phases of archaeology from the very beginning of the history of the Carnegie Institution. In the earliest years, indeed, programs were formulated and grants were made for archaeological research in a wide variety of fields and a wide range of geographic locations. It was in 1913, however, with the publication of *Reports upon the Present Condition and Future Needs of the Science of Anthropology*, that a direction of research was outlined and crystallized which was to set the main course of effort of the Institution in this field for the succeeding forty-five years—an approach determined largely by the able advocacy by Sylvanus G. Morley of the Maya civilization of Middle America as an appropriate field of concentration.

With the approval of that course by the Trustees of the Institution there followed a preparatory phase of exploration, reconnaissance, and planning which occupied ten years. It was a fitting introduction to the intensive period of field research that followed. Explorations in the Peten region of Guatemala, in Honduras, Nicaragua, and Costa Rica were accompanied by preliminary investigation of the ruins of Copan and were crowned by the discovery of the ruins of Uaxactun, with “the oldest monument yet reported from the Maya field” in 1916 and the publication of Morley’s important monograph on the inscriptions at Copan in 1920. This exploratory period was characterized by another notable feature, the significance of which to further research it is hard to overemphasize—the development of two basic principles of procedure in field work. First, the Institution in its research programs should refrain from exporting any of its archaeological finds from the countries of their origin but rather should return all of them to the appropriate government authorities when study was completed. Second, the Institution should assume an obliga-

tion to preserve remains, once uncovered, from the further deterioration by weathering which would otherwise be inevitable. This philosophy defined a climate that has been maintained throughout the entire program of archaeology of the Institution—a tone that has fostered and expanded a continuing measure of good will.

It was in 1924 that large-scale excavations at Chichen Itza in Yucatan, recommended in Morley's original report, were begun in earnest, to be continued unabated for ten years. Two years later investigations of similar intensity were begun at Uaxactun and carried forward for a dozen years thereafter. This was the period of the most intensive exploration and discovery—the period, possibly, when our vision and knowledge of the true nature of the complex of Middle American cultures were most rapidly expanded and brought into focus. In 1928 George C. Vaillant, in the course of a visit of but a few days to Uaxactun, sank a pit through the various plaza levels of the ruin into the soil beneath. The artifacts that appeared from this level were not Mayan in the accepted sense, but rather strongly suggested the early Archaic pottery of the Mexican and Guatemalan highlands. This important discovery opened a whole new horizon of Maya prehistory, suggesting a major concept then quite new—the idea that there might have been connections between the cultures of the lowland and the highland Maya regions at a very early period—that indeed there might have been, in some sense, a common base linking them in early pre-Classic Mayan times. That concept led on to a program of extensive investigation in the Guatemalan highlands, begun in 1932 and culminating in the pivotally important excavations of two inconspicuous mounds at the great ruin site of Kaminaljuyu outside of Guatemala City by Alfred V. Kidder, Jesse D. Jennings, and Edwin M. Shook in 1936, 1937, 1941, and 1942. That work resulted in a linkage of the great Classic centers of Middle American culture in the Valley of Mexico, the Valley of Oaxaca, the Guatemalan highlands, and the lowlands of Peten. For the first time, the high cultures of Middle America were brought into focus in time and space, and it was revealed that they formed one great area of relatively homogeneous, or in any case interdependent, civilizations. It was established that the Mayan Classic civilization had a base far broader and deeper than earlier workers had imagined, that it was not a unique development from which, alone, high culture was disseminated to adjacent peoples; that, on the contrary, the Classic phases of a number of other cultures were roughly coeval with the Maya.

Perhaps this demonstration of the homogeneity and the massiveness of the Middle American cultures, of the breadth of the common base upon which they rested in the cultures of the Formative period, and of the age, the complexity, the high development characteristic of these Formative cultures themselves—emphasized particularly by later excavations at Kaminaljuyu in the

period 1946-1950—represented among the most important of all the results of the Mayan program in the Institution. That situation was further underlined by the finding of sites of Formative period pottery in the northern part of the Yucatan peninsula in 1942 by G. W. Brainerd, proving that the northern cultures were as deeply rooted as the southern, and that the relatively specific sequences between the cultures of the north and south which had earlier been visualized were no longer tenable. The massiveness was emphasized by the final major field program of the Department, undertaken in 1950, at the site of Mayapan, the last great aboriginal Mayan city. This was an intensive investigation, carried forward in depth, with particular emphasis on the secular aspects of the culture. It established the time of the greatness of Mayapan beyond reasonable doubt. It also established another finding of singular importance. There had been a widespread assumption that the final and dramatic break with cultural tradition that marked the close of Mayan greatness—the precipitous descent from order and high culture to what must have been chaos bordering on ruin—occurred with the rise to power, under foreign domination, of the great center of Chichen. The investigations identified that precipitous decline rather with the end of the greatness of Chichen and the rise to power of Mayapan itself.

These are but the broad and blurred outlines of an extensive and varied program. In the very wealth of its content, it is easy to overlook facets somewhat separated from the main stream but of intrinsic importance, such as the program of study of the Early Basketmaker II culture of the North American Southwest, carried on for ten years in Arizona, New Mexico, and southern Colorado by Earl H. Morris, with very interesting results. It is easy to overlook special but central aspects of the work, such as the long and fertile history of research in Mayan hieroglyphics and the investigations in ceramics, or dramatic specific findings, such as those resulting from the excavations at Copan or the spectacular discovery of the famous wall paintings of Bonampak, in the lowland Maya country. Finally, it is all too easy to neglect or underestimate one of the most important consequences of the whole program of Middle American research in the Institution: the cordial relations that were established with the countries in which the investigations were made, the good will that was uniformly generated, and the great stimulus to local efforts of research in the field that the program conferred. The significant strengthening of the archaeological interests and programs of the Mexican Government, and the establishment of a museum of archaeology and ethnology by the government of the Republic of Guatemala and the organization of a national institute of anthropology and history there, represent but two of the tangible consequences of this policy and the climate it created and maintained.

The record of the Department of Archaeology which closes this year has been

a great one, worthy of special pride. It is a particular satisfaction that its closing does not mean that all effort in this field will be terminated. The work of the Institution will continue to be enriched by the investigations of the Director of the Department and of certain members of its staff.

The Department of Terrestrial Magnetism has continued its active program in radio astronomy, the study of the emission of energy at radio frequencies by celestial objects, of which the Crab Nebula provides so striking an example. The past year has witnessed solar activity of unprecedented magnitude. This has interfered seriously with optical redshift measurements of the far distant galaxies at Mount Wilson and Palomar. It has also made it necessary, as was mentioned last year, to postpone for the time being the continuing program of measurements of radio frequencies from astronomical objects in the range of 12 to 15 mc which was begun at the Department of Terrestrial Magnetism in 1955-1956. Work has, however, proceeded rapidly on precision equipment designed to measure the position of radio sources to within a few square minutes of arc. This is a 400 mc/sec linear array, consisting of a pair of V-reflectors, each 614 feet long, arranged on an east-west baseline with a spacing of 1842 feet between centers. These arrays can be used separately, when they yield a fan beam $\frac{1}{2}^\circ \times 20^\circ$ to half-power points, or they can be employed together as an interferometer with a lobe spacing of 4 minutes of arc. Calculations indicate that the two arrays should have an effective collecting area equivalent to that of two 90-foot paraboloids. At the close of the year, all the elements of this new system had been assembled and mounted and final connections were being made. A test had also been completed on the phase stability of open-wire transmission lines. This is a factor vital to accuracy in the final measurements. The limits with respect to the conditions of temperature, humidity, and frost under which the system will operate appear, as a result of tests, to be such that the system will be operable for a considerable fraction of the time—perhaps as much as three-quarters of the total.

Last year an antenna array specially designed for a detailed examination of the radio emission of the sun was built by the Department at its River Road site near Seneca, Maryland, and preliminary scanning of the sun's disk revealed localized bright sources which traveled across it as the sun rotated. By the end of this year, the equipment had been operated long enough to provide a good description of the events occurring on the sun at a wavelength of 340 mc. The most common features found were quiet bright spots, already detected and illustrated last year. Their positions on the disk usually agreed with that of large plagues, but the converse was by no means always true—other, equally large, plagues seemed to have no radio bright spots associated with them. The spots persisted for several days, sometimes for an entire disk passage.

A second common feature of the solar face observed at 340 mc consisted of active spots which, though persisting for several days like the quiet spots, showed changes in intensity and produced frequent small bursts lasting a second or less. Active spots may be much more intense than the quiet ones; the largest detected so far gave a steady flux of 60×10^{-22} watt/meter²/cycle/second, with instantaneous values of perhaps twice this magnitude. These values are to be compared with fluxes from about 5×10^{-23} watt/meter²/cycle/second (the smallest that can be detected) to about 5×10^{-22} watt/meter²/cycle/second for the quiet bright spots.

At the close of the International Geophysical Year, it is interesting to recollect that the idea actually took its origin in discussions among several members and former members of the staff of the Department of Terrestrial Magnetism in 1950. There had been a half-century interval between the two previous International Polar Years (1883 and 1933). It was generally understood that the next period of large-scale international collaboration in geophysical studies would come in 1983. But the tremendous advances in exploration made possible by modern radio communication and techniques of air reconnaissance and airlift supply suggested that a shorter interval of 25 years would be especially attractive and appropriate. A suggestion to this effect at an international meeting of geophysicists led quickly to wide approval and support.

It seemed most appropriate, therefore, that the Department participate in the world program which it did so much to initiate, and that, in accordance with the philosophy of the Institution as a whole, this participation be on the basis of active individual research. Accordingly, three noteworthy projects have been carried forward during the past year as a part of the IGY program. All of them represent further extensions of interests already developed in the Department.

The first of these projects concerned the study of the intense band of electric current, called the "electrojet," that circulates in the upper atmosphere in the region of the earth's magnetic equator. Such a study is of peculiar interest during periods of magnetic disturbance. Four Askania variographs loaned to the Department through the cooperation of the U. S. Coast and Geodetic Survey were put in operation at temporary magnetic observatories established on the coast of Peru at Talara, Chiclayo, Chimbote, and Yauca, extending from geographic latitude 4° N. to 15° S. The project was initiated in collaboration with the Instituto Geofísico de Huancayo. A permanent magnetic observatory was also established for the University of Arequipa by the Instituto Geofísico for which the Department provided a la Cour vertical intensity variometer. In this multistation network spaced on both sides of the magnetic equator in Peru the "electrojet" phenomenon has been extensively investigated during the year and magnetic storm and disturbance relationships have been studied.

The second project in which the Department has participated with the IGY concerns a field far removed from considerations of galaxies and stars but of even more immediate concern and appeal—the nature of our own earth. For many years the Department has been conducting an extensive series of seismic and gravity studies designed to increase our understanding of the structure of the earth's crust, and especially of large-scale processes which, operating over long periods of time, have resulted in the formation of continents and ocean deeps, high plateaus and mountain ranges. In previous years this program has included field studies on the Colorado Plateau and in Alaska. Last year it was reported that a new study, of similar nature, was planned in the Andean highlands, utilizing explosions normally set off in the operation of large open-pit copper mines. During the past summer this was carried out, with results both interesting and somewhat puzzling, which are at present under study. In the high montane and alpine plateau regions of Peru, Bolivia, and Chile some indication was obtained that the crustal thickness under the Andes may be 10 to 20 km greater than had previously been found in North America. Unusually severe attenuation of the reflected waves was encountered under the high Andean plateau, however, and continuation of the investigations, probably by earthquake observations in collaboration with university colleagues in Peru and Chile, is anticipated.

The third area of interest to the Department which was expanded and intensified over the past year within the framework of the IGY is one which it has long shared with the Geophysical Laboratory—the measurement of the ages of rock minerals through the methods provided by the study of radioisotopes. Collections of samples for this work were made in South America, northern Europe, and selected additional regions of the United States.

The program on mineral ages conducted by the cooperative age group, consisting of L. Thomas Aldrich and George W. Wetherill in the Department and George R. Tilton and Gordon L. Davis in the Geophysical Laboratory, has been concerned this year with investigations of the geographical patterns of the ages of Precambrian rocks in North America. A comprehensive study of findings made by the Institution, together with data obtained at the geology departments of the University of Minnesota and the Massachusetts Institute of Technology and at the Lamont Geological Observatory, has developed a rather definite picture of the broad outlines of major periods of mineral formation for those parts of the continent that have now been measured. A large area with rocks of ages exceeding 2500 million years extends from western Quebec through Ontario to eastern Saskatchewan and northern Minnesota. It reappears in Montana and Wyoming. A second large area, with rocks close to 1850 million years old, occurs in the western United States. Rocks of this age are now known also from Missouri, Wisconsin, Michigan, and Ontario. A third large region having similar-aged rocks extends from northern Quebec south into

Ontario, New York, New Jersey, Virginia, and North Carolina. All these rocks were formed close to 1000 million years ago. Thus this period too appears to have been one in which active processes of mineral formation were proceeding over large areas in North America.

The existence of five different nuclear clocks (decay of U^{238} , U^{235} , Th^{232} , Rb^{87} , K^{40}) has created new opportunities for research on the time sequences of ancient processes, a second major concern of the cooperative age group. A whole series of pertinent questions may now be fruitfully asked about typical mountain chains, such as the Appalachians. How old are the ancient crystalline rocks that predate the orogeny? How did the Paleozoic upheaval, occurring 300 million years ago, affect the apparent ages of minerals in the old rocks? What was the sequence of events in various parts of the belt?

Intensive study has been given to the metamorphic Precambrian rocks of the central and southern Appalachians. Measurements of mineral ages and petrographic examination of the gneisses of this region suggest the possibility that there have been two major periods of mineral formation. During the first, dating from 1000 to 1100 million years ago, zircon and probably potassium feldspar were formed. The age of zircon from the Baltimore gneiss, a part of the basement complex of the Appalachian Piedmont, has been determined as 1100 million years from nearly concordant uranium-lead ages. During the second period of active mineral formation, which occurred about 300 to 350 million years ago, it would appear that the mica of the gneiss was formed. Biotite ages of about 300 million years have been established by both rubidium-strontium and potassium-argon methods. It appears that the rock may have crystallized about 1100 million years ago and that the biotite age is related to local metamorphism taking place some 800 million years later.

A general picture of geologic history of the Appalachian orogenic belt emerges. The belt, extending along the Atlantic coast from southeastern Canada to the southeastern United States, apparently experienced several periods of deformation between 250 and 400 million years ago. Great thicknesses of sediments may have been deposited on a basement of gneisses and granites. Subsequently both sediments and basement sank into the crust and were subjected to elevated temperatures and pressures. Here some of the crystalline rocks and sediments were altered to metamorphic rocks, and granitic rocks were at the same time formed or intruded. Finally, uplift and erosion exposed the resulting assemblage, now including gneiss, schist, marble, quartzite, and granite.

Studies of mineral ages during the year have not been confined to rocks of the North American continent, or even of the western hemisphere. During the Andes expedition of the Department, as indicated earlier, samples of rocks were obtained from Peru, Chile, and Brazil. Peruvian specimens have now been analyzed. The evidence suggests that they are Paleozoic. Zircon obtained

from a gneiss at Koli, Finland, which occupies a position there analogous to that of the Baltimore gneiss, was studied for comparison with the Appalachian samples. It presents an interesting discordance. Thus rubidium-strontium and potassium-argon determinations by J. A. O. Kouvo of biotite from several gneisses in the area suggest an age of about 1800 million years, while the Pb^{207} - Pb^{206} determinations suggest an age for the zircon of 2600 to 2700 million years. Possibly the gneiss had an age of 2600 to 2700 million years and was metamorphosed some 1800 million years ago.

When the ages of all the principal regions of rocks underlying the whole of the North American continent have been determined, it may be possible to evolve a critical picture of the way in which the continent was formed. A principal question will be whether the continent has grown from an ancient "nucleus" by the successive addition of materials derived from depth during successive orogenies or whether it has always had a considerable area. The clarifying of this picture forms one major goal of the studies of the ages of rocks in North America.

Investigations yielding quite different kinds of information bearing on the processes of mountain building continue to be prosecuted vigorously in the Geophysical Laboratory. They are concerned with the behavior of mineral constituents under conditions of temperature and high pressure approximating those obtaining deep within the earth's crust. To this end, new equipment of rather revolutionary design for studying geochemical and geophysical phenomena has been constructed at the Geophysical Laboratory by Francis R. Boyd, Jr., and Joseph L. England, and is currently under test. Pressures approaching 100,000 atmospheres have been attained at temperatures of 300° to 400° C with the "squeezer" equipment described in last year's report. It is desirable, however, to work at much higher temperatures. With the new "single-stage" apparatus, pressures of 50,000 atmospheres combined with temperatures up to 1700° C have been achieved. With still newer equipment, designated as the two-stage design, in which the piston of the single-stage apparatus is supported, successful experimental runs of 65,000 atmospheres' pressure have been made at a temperature of 1100° C. The single-stage equipment is being used to study the influence of pressure on the melting points of minerals that might exist 80 miles below the earth's surface.

Data on a number of systems are beginning to accumulate. Of particular interest is information on the change in melting point of various silicates with pressure—data of special importance since they define a limit to the geothermal gradient within the earth's mantle.

Studies of phase equilibria are being continued in other connections. Hans P. Eugster and Dr. Charles Milton, of the U. S. Geological Survey, have been interpreting phase-equilibria data on alkaline carbonates to explain some of the

bizarre mineral assemblages of the Green River shales of Colorado, Wyoming, and Utah. These shales, of Eocene age, contain the world's largest reserves of hydrocarbons. Associated with them are a number of unusual alkaline minerals, such as trona ($\text{NaHCO}_3 \cdot \text{Na}_2\text{CO}_3 \cdot 2\text{H}_2\text{O}$). This highly soluble salt is found in a bed 10 feet thick and several miles wide. If ordinary river water is concentrated by evaporation in a large lake basin, an alkaline solution results. Trona is known to be precipitated above about 35°C from concentrated brines in equilibrium with air containing 300 to 400 parts per million of carbon dioxide. The formation of trona in Wyoming can be explained by the evaporation of brines, in a shallow warm basin, equilibrated with air having approximately the same carbon dioxide content as that of the present atmosphere.

Geothermometry continues to be of great interest also in quite another context: the study of ore deposits. Ore minerals have been under intensive investigation during the year in the Geophysical Laboratory by Gunnar Kullerud, R. G. Arnold, H. L. Barnes, L. A. Clark, and E. H. Roseboom, Jr. More than a score of systems are under study, the data from which will ultimately be applicable to ore-deposit geothermometry.

Hatten S. Yoder has continued and expanded his studies on the effect of water on the melting relations of rock-forming silicates, while Eugster, D. R. Wones, and A. C. Turnock have studied pressure-temperature stability characteristic of hydrous iron-bearing micas and chlorites.

Investigations in experimental petrology have made active progress during the year. They include a study of cordierites by Yoder, J. Frank Schairer, and W. F. Schreyer, of pyroxenes by Schairer and N. Morimoto, and of amphiboles by W. G. Ernst, and a comparable study of spinels by Wones and Turnock. A particularly interesting accomplishment has been the synthesis of a low-temperature form of cordierite identical with samples of the natural mineral obtained in Albany County, Wyoming, and Guilford, Connecticut.

It will be recalled that one of the high points in the research of the Geophysical Laboratory recorded last year was the work of Felix Chayes, in which optical analogues were developed to simulate diffraction patterns obtained in X-ray studies of crystal structure. By varying the analogue, Chayes was able to study the kinds of patterns that might be obtained in various types of order-disorder in crystals, laying particular emphasis on the "short-range" ordering concerned with nearest-neighbor pairings. This approach has been continued, refined, and expanded during the current year. Developments have concerned the design of random-layered sequences characterized by arbitrary levels of short-range ordering, the production of experimental diffraction masks based on such sequences, the generation of diffraction transforms from these masks, and direct calculation of the diffraction effects by high-speed computation.

The Geophysical Laboratory has for a number of years been conducting a

vigorous program in crystallography, of central importance to many fields of research. Almost forty-one years ago the first account of a crystal structure to have been determined in the United States was published from the Throop College of Technology, the parent of the California Institute of Technology. It was a determination of the structure of chalcopyrite, and it was made possible by a grant from the Carnegie Institution of Washington to A. A. Noyes, at whose suggestion the research was undertaken. It is therefore of particular interest that this year an intensive investigation of the nature of chemical bonding in chalcopyrite by neutron diffraction procedures has been undertaken collaboratively by G. Donnay, J. D. H. Donnay at Johns Hopkins, and Drs. L. M. Corliss, Julius M. Hastings, and Norman Elliott at the Brookhaven National Laboratory. The data on structure already available from X-ray diffraction studies could not give complete information on the nature of the bonding. The neutron diffraction work has now measured the magnetic properties of the mineral. Interpretation of the data indicates that the substance is best represented by the formula $\text{Cu}^+\text{Fe}^{+++}\text{S}_2$. In an associated research Morimoto has conducted a crystallographic study of arsenopyrite, one of the common sulfide minerals, using X-ray diffraction methods.

During the year Willard F. Libby has carried on a series of researches on the geochemistry of fission products. In particular he has been concerned with practical aspects of soil contamination with strontium 90 and the ways in which it may be alleviated. It has been shown that potassium, when introduced into soil at as low a level as about 60 pounds per 2 million pounds of soil, reduces the observed uptake of radioactive strontium in experimental plants by something like 40 per cent.

The Geophysical Laboratory has made particular efforts to counteract a situation that plagues scientific research everywhere, but is perhaps unusually serious in geochemical and geophysical research. Publications in these fields are so widely scattered that many of them must be effectively lost to most graduate and professional students of the earth sciences. A particularly significant contribution to the alleviation of this problem was made during the academic year 1957-1958 in the holding of a series of weekly seminars and discussions on the topic "Researches in Geochemistry" at the Laboratory and at the Johns Hopkins University. The participants included some of the leading geochemists of the country. The manuscripts have been edited at the Laboratory and will be issued as a symposium volume early in 1959. Much new work, as yet unpublished, will be included.

Few aspects of modern research are more challenging than those of theoretical biology. The Carnegie Institution is deeply concerned with this frontier. Five of the seven departments include in their programs investigations in the life sciences. They range in aspect from the organization of molecular units

in vital processes through the nature and function of somewhat larger but still microscopic or submicroscopic intracellular entities like cell walls, microsomes, chromosomes, ribonucleoprotein particles, and chlorophyll-protein complexes, to the processes underlying the differentiation and organization of cells themselves in the developing many-celled embryo, and finally to the structure and interaction of natural populations of many-celled organisms, with the questions of experimental taxonomy and of evolution which they invoke.

In the Geophysical Laboratory Philip H. Abelson has this year investigated the detection of organic materials in Precambrian rocks. Shales and slates of a low grade of metamorphism like the Huronian Rove slate and the Keeweenawan Nonesuch shale have been examined. Yields were very small when extraction methods using an ethyl alcohol-benzene mixture in a Soxhlet apparatus were applied to the latter and negligible for the former. Exploratory experiments involving hydrogenation, carried out on Swedish Kolm shale, of Cambrian age, Nevadan Vanini shale of Ordovician age, and Colorado Green River shale of the Eocene, showed that this method is capable of increasing the organic yields from these processed shales very significantly. Values as high as 70 per cent of the total organic matter present in the Kolm shales were obtained, in contrast to only 1.6 per cent obtained from an aliquot sample extracted without heating, and 8 per cent from another sample extracted after heating at 375° C for 5 hours in a nitrogen atmosphere. Hydrogenation, therefore, is very effective in rendering organic matter more extractable, and may have an important application in the difficult problem of investigating organic material in the Precambrian sediments. The approach seems attractive enough, indeed, to encourage further investigation of its applicability to studies of organic sediments of all ages.

Studies particularly concerned with tracing the pathways of synthesis of proteins and nucleic acids in microorganisms have been under way in the biophysics group of the Department of Terrestrial Magnetism for a number of years. Recently attention has been focused particularly on the critical importance in metabolism and growth of the elaborate and precise fine structure of many of the constituents of living cells. Among these constituents, the lipoprotein membranes and ribonucleoprotein particles which together comprise the microsomal fraction seem of special significance and interest. Such entities, which range from molecular weights of one to four million, appear to be essential in the synthesis of protein. They are under intensive investigation.

It already seems clear that particles of this type, obtained from the bacterium *Escherichia coli*, are complex structures, composed of ribonucleic acid and a number of different proteins held together by hydrogen bonds. One of these proteins is the enzyme ribonuclease, which is inactive while held within the particle but when released by breakage of the hydrogen bonding can exert its

enzymic capacity, hydrolyzing the nucleic acid. In the analytical centrifuge the particles show a spectrum of sizes. The distribution of particles within that spectrum, interestingly enough, depends strongly on the rate of cellular growth. Bacteria rapidly growing in a broth medium contain more of the smaller particles than bacteria growing in a glucose-salt medium, and cells that have been treated with chloramphenicol to halt protein synthesis contain mostly one class of ribosome. These findings suggest that the different sizes may correspond to successive stages of formation of the particles and that the spectrum of ribosome size distribution can serve as an indicator of the protein-synthesizing ability of the cell. Studies using the incorporation of tracer molecules indicate that the larger particles may be formed by incorporating protein and nucleic acid macromolecules. These early results emphasize that an understanding of the role of nucleoprotein particles may constitute a major step in working out the mechanisms of the synthesis of proteins and nucleic acids.

A very different approach to the problem of protein synthesis has been provided by the use of amino acid analogues. It will be recalled that last year an investigation carried out in collaboration with Dr. Georges N. Cohen of the Institut Pasteur showed that selenomethionine can replace methionine and support exponential growth in a methionine-requiring mutant of the bacterium *E. coli*. Study of such analogues has been continued extensively during the year in collaboration with Dr. Cohen and with H. de Robichon-Szulmajeter of the National Institutes of Health. Particular emphasis has been laid on determining whether the analogues are contained in radically different molecular species or in proteins similar to those normally synthesized. Such investigation requires analogues that will substitute for only one naturally occurring amino acid. Norleucine, which substitutes for methionine in the proteins of *E. coli*, has proved especially useful for this purpose.

The proteins incorporating the analogue appeared to be only slightly altered. Furthermore, analogues are incorporated into different proteins in the same proportion, suggesting that the mechanism for amino acid selection does not differ from one protein to another. With some analogues, enzymes retained their usual biological activity; with others, they were inactive. This observation suggests that the amino acid complement of the active sites of an enzyme can be examined by studying the sensitivity of the enzyme to a spectrum of analogues. Analysis showed that each of the many proteins that could be resolved by ion-exchange chromatography incorporated the analogue to the same extent. There thus appear to be no differences in selectivity among the mechanisms that make up the different proteins. Such a result would be expected if the selection of the amino acid is determined by the order of the nucleotides in ribonucleic acid.

One of the central concerns of the Department of Plant Biology has long been the nature and mode of action of chlorophyll as it occurs in living plants—as it is involved in that economically supremely important natural reaction, photosynthesis. Early in this century it was hoped that the mode of action of chlorophyll in photosynthesis would become evident, once its chemical structure was established. Time has proved that the problem is far more complex and subtle than this. When knowledge of structure did not establish an adequate basis for comprehending the mechanism, it was hoped that isolation of functional units of chlorophyll and examination of their properties would greatly aid an understanding of chlorophyll action. It turned out that functional units of active chlorophyll with simple proportions of pigment and protein were not isolable from mature plants.

A much more fertile line of attack was developed a number of years ago by James H. C. Smith at the Department in the study of the freshly formed chlorophyll complex. This chlorophyll-protein complex, known as the chlorophyll holochrome, seems to be a definite chemical individual of the protein-prosthetic group, isolable in particles of closely uniform size, and amenable to study outside the green leaf. Investigation of its properties has occupied an important place in the work of the Department for a number of years.

The search for an explanation of the very different properties of chlorophyll in the natural complex and in its pure form continues to be very much in the foreground. A basic problem is to establish the range of variation of the measurable properties of the natural chlorophyll-*a* complex. How many recognizable forms of chlorophyll *a* exist in plants?

It is quite generally believed that two different natural forms of chlorophyll *a* occur together in most green plants. Neither has been isolated as a chemical substance, their existence being deduced from the relative efficiency of photosynthesis at different wavelengths of light. The derivative spectrophotometer designed and built several years ago in the Department by C. Stacy French and his colleagues has proved a particularly useful tool for this investigation. An extensive survey has been undertaken during the year just past of the derivative absorption spectra of chlorophyll in numerous algae and other plants. The survey seems to indicate that, contrary to the common assumption, there must be more than two forms of chlorophyll *a*. One new form, very different from those previously recognized, has an absorption maximum at the very long wavelength 695 mμ. It is found in old—but not in young—cultures of the green alga *Euglena*. It appears to be chlorophyll *a* combined in an unusual complex.

Within the last two years there has been developed in several laboratories a physical concept of the mode of action of the natural chlorophyll complex that may ultimately prove one of the most illuminating to our understanding

of photosynthesis. A pioneer in the development of these ideas has been Dr. William Arnold of the Oak Ridge National Laboratories, and the second Fellow of the Carnegie Institution under the program made possible by the Carnegie Corporation. The concept envisions the functional units within a chloroplast as in effect photocells whose behavior in energy trapping and energy transfer can be considered in terms of the theory of semiconductors. Few departures in the field of theoretical photosynthesis in the past few years have been as radical, as provocative, or as suggestive as this. It has already led to several kinds of new experiments and provides a description in terms less specifically related to chemical structure than other models that have been attempted.

The investigations of Smith on the photochemical formation of chlorophyll in leaves from its precursor protochlorophyll, carried on for many years, have been actively continued. This year attention has been focused particularly on the amount of light required to form a molecule of chlorophyll. Protochlorophyll with its carrier protein can now be removed from dark-grown leaves in active form and purified to a reasonable degree. The absorption coefficients of protochlorophyll and of chlorophyll *a* are known within a few per cent. It seemed feasible, therefore, to measure the efficiency of the photochemical conversion of protochlorophyll to chlorophyll. Extracts of protochlorophyll were illuminated, and the incident light absorbed by active protochlorophyll in the solution was calculated. After the exposure the amount of chlorophyll formed was measured spectroscopically. The yields ranged from about 0.5 to 0.7 molecule of chlorophyll formed per quantum of light absorbed, with an average of 0.6 molecule. It is tempting to think that two quanta of light may produce one molecule of chlorophyll. Experiments are being continued and methods improved to obtain more precise values.

In attempting to elucidate the situation in the higher green plants a comparison of the characteristic chlorophyll with the chlorophyll in the purple bacteria that are capable of photosynthesis is particularly useful. An interesting investigation of the reversible oxidative bleaching of chlorophyll by chemical treatment and by light in several species of photosynthetic bacteria has been undertaken during the year by J. C. Goedheer. The study has shown that the chemical properties of the pigment are markedly influenced by its incorporation in natural structures. Several coexisting forms of bacteriochlorophyll have been found to have differing chemical reactivity. The form that absorbs light at the longest wavelength (890 m μ) is the most active. This is the form that L. N. M. Duysens earlier discovered to be the receiver of energy absorbed by other pigments; it is also the fluorescent form. The fact that the absorption bands of the different forms of chlorophyll in living purple bacteria are so much more widely separated than in the green plants makes them peculiarly advantageous as a reference point in work of this kind.

Possibly there are no more fundamental aspects of theoretical biology than those involved in the study of genetic mechanisms. They underlie the whole field, and so must be of essential concern to each of the departments occupied with biological matters. Questions involving the structure and function of the materials of heredity have, however, occupied a central position for many years in the research of the Department of Genetics. In the course of this work many different organisms have been and are currently being employed: maize—the plant which more than fifty years ago enabled G. H. Shull in the Department to work out the basic principles of hybrid vigor; the fruit fly *Drosophila*, long a classic tool of geneticists; and more recently and more especially, those most modern tools of genetics, the usefulness of which has been adequately recognized and exploited only in very recent years—bacteria and viruses.

In the field of virus genetics, Alfred D. Hershey has been conducting researches of great interest, designed especially to elucidate the mechanisms of heredity in certain strains of bacteriophages. During the past year his experiments, and those of his co-workers, have shown that both genetic recombination and chromosomal replication take place in bacteriophages in the absence of protein synthesis. On the other hand, quantitative analysis of the effects of ultraviolet light on genetic recombination strongly suggests that such recombination is directly linked to the synthesis of deoxyribonucleic acid.

For several years M. Demerec and his group have been deeply concerned with what may be called the “fine structure” of the bacterial chromosome, as investigated by the methods of biochemical genetics. It is now possible to analyze the structure of certain segments of the chromosome concerned with biochemical syntheses to such a degree of resolution as to distinguish active “sites” located not more than a few wavelengths of light apart. An important test object in this research has been the bacterium *Salmonella typhimurium*, the agent of mouse typhoid. Stocks of this organism have been constructed embodying combinations of genetic markers mediating the metabolic incorporation of a number of amino acids, such as cystine and tryptophan. Particular use has been made of the phenomenon of transduction, now well known. A fragment of bacterial genetic material, incorporated within an infecting particle of bacteriophage, can be carried by this phage to a second bacterial host and there incorporated intact into the new genetic complex. It is frequently possible for the larger bacteriophages to carry a fragment of bacterial chromosome long enough to include a number of linked and separately identified metabolic sites. This situation offers the opportunity of presenting the receiving bacterium with a dilemma. It can be made to include within its genome a pair of sites—or a site and its recessive allelomorph—at points where it would normally contain but one. The question then arises which of the two alternative sites will be perpetuated when the bacterium divides: which, in short, will be reproduced. This phenomenon, known as “copy choice,” is of great interest

in itself, but it is also important because of the light it sheds on the detailed mechanism of chromosome replication.

By means of such studies, using five linked genetic markers that can be carried together in one transducing fragment, Demerec and his group have this year obtained information suggesting that a definable copy-choice mechanism operates during the formation of the recombinant offspring of an infected bacterium. According to their interpretation, recombination occurs at the time of chromosome replication, when, presumably, a transduced fragment is closely synapsed with the homologous region of the chromosome of the recipient bacterium. Chromosome replication proceeds by the formation of copies of small parts of the chromosome and the joining of these replicas in a zipperlike manner. In the duplicated regions, replicas may be modeled either on the site already present in the bacterium or on that received in the transducing fragment. But, once a choice has been made—after the first replica of one member of a duplicate set has been formed—the choices for subsequent replicas do not occur at random. There is a higher probability that consistency will be maintained—a better chance that, however the choice was made on the first replication, so it will remain thereafter. The experimental data indicate that the frequency of occurrence of nonconsistent switches depends on the genetic constitution of the chromosome regions involved. These findings may prove of great importance to our understanding of the detailed mechanisms by which that most fundamental of all the properties of life—the duplication of chromosome material—occurs.

In a related research directed to a slightly different end, Allan Campbell has been investigating a transduction system in which genes of the bacterium *Escherichia coli* concerned with the metabolism of galactose are carried from one cell to another by the bacteriophage lambda. Earlier work had indicated that the crucial intermediate in this process is a hybrid genetic structure containing information derived partly from the phage and partly from the bacterial host. The work of the past year has strengthened this hypothesis. An array of such hybrid structures has been accumulated, each arising from a separate primary transductional event, and they have been found to differ significantly in the exact content of genetic loci. Thus some of the details of the whole process are becoming clearer.

The remarkable program of Barbara McClintock in the genetics of maize, which has been in progress for a number of years, is continuing, with new and exceedingly interesting findings. It will be recalled that this work involved the detection, and the subsequent investigation, of systems of elements in the cell nucleus that control the action of particular genetic loci without themselves taking a specific part in the shaping of cell function or morphology in the manner classically associated with the action of genes. Last year the finding of

a system was reported which controls gene action at two known loci, not directly related to the *Ds-Ac* system earlier described.

One of the consequences of this situation, earlier discussed, is that the activity of a particular gene may vary during the development of a maize plant. Thus the mature plant may exhibit entirely different patterns of expression of the same gene in its various parts—a matter of the greatest interest to those concerned with cell differentiation in the many-celled embryo. In some cases, both the pattern type and the inheritance of pattern type in subsequent progeny appear to be very irregular. Analysis of one such case this year has revealed the underlying mechanism clearly. The complexities were found to be a result of alternating cycles of action and inaction in one particular controlling element, resulting in changes in expression of the gene. Further analyses of actions of this type can be of the widest biological importance.

The Department of Genetics shares with the biophysics group in the Department of Terrestrial Magnetism an intense interest in the intracellular deoxyribonucleases. A proper understanding of their properties seems essential to a solution of general problems concerned with the metabolism of deoxyribonucleic acid and its role in cell division. During the year Margaret R. McDonald has continued her studies directed toward the isolation and characterization of these enzymes. Last year she discovered that salmon testes offer an important natural source of experimental material. Her efforts this year have been concentrated on ascertaining the best procedures for extracting and purifying the deoxyribonuclease of these tissues. An enrichment of activity of more than six hundred fold (measured in units per milligram of protein) has been attained without any appreciable loss in total enzymatic activity. The best product so far obtained, however, is not homogeneous. It is composed of at least three proteins. One has been crystallized, but not yet analyzed for biological specificity. Methods are at present under study to permit still further purification of the deoxyribonuclease from salmon testes, in anticipation of a detailed study of its properties and its mode of action.

The level of biological organization involving the combining of cells into tissues, and the problems of cell differentiation, regulation, and coordination that they involve, are the special concern of the Department of Embryology. Research in the Department during the year has proceeded most actively and on so many fronts that only a few can be cited here.

Countless students of biology have been intrigued by the movements of spermatozoa. It is clear that motility may be ascribed to processes occurring in the sperm tail, involving a synchronous cycle of contraction and relaxation. In studying the basis of this rhythmic motility, David W. Bishop has made what may prove to be an important advance in the discovery that the mechanisms of contractility and coordination can be experimentally disengaged, thus permitting separate analyses of the processes. The extraction of mammalian

sperm with glycerine divests them of their normal properties of permeability and irritability. Simultaneously they lose their ability to beat in a coordinated, spiral manner. Such cell models beat rhythmically, but only in one plane. Their only forward movement is slow and jerky, and occasionally they may even move backward. But the two-dimensional beat is vigorous and may actually be of higher frequency than that of the normal unextracted sperm. These extracted cells have thus retained their mechanism for contraction, but have lost the coordinating mechanism that normally provides for a contraction sequence in adjacent fibrils.

On the other hand, unextracted spermatozoa, cells with relatively long and flexible tails like the sperm of the common squid, can be slowed down by dilution of the sperm mass or by the gradual depletion of the energy reserves of the cell and the substrates that supply these reserves. In one sense this treatment "uncouples" the two kinds of sperm motility. Stationary cells, and even those drifting backward in the medium, can rotate rapidly about their longitudinal axes, owing to a spiral flagellation of the tail elements. The frequency of this type of fibrillar motion is high, and, for any given sperm, the direction of rotation remains constant. The second, more violent, but less rhythmic beat in these retarded sperm is a two-dimensional lashing of the tail. This contraction-relaxation cycle has a time constant distribution of about three to one, with a definite pause intervening between contractions. The experimental separation of the contraction-relaxation mechanism from the coordination mechanism raises a number of provocative questions and clearly constitutes a problem demanding further attention.

James D. Ebert has recently presented an analysis of progress in the field of immunoembryology under the title "The acquisition of biological specificity"—a title reflecting one of the principal themes of research in the Department. It is concerned with the mechanisms of synthesis and interaction of specific macromolecules. It embraces problems of tissue specificity and individual specificity. Although tissue specificity may, in part, reflect differences in associations and numbers of macromolecules which are alike in all tissues, immunochemical and physiological techniques are capable of distinguishing molecular types characteristic of each tissue. The difference between two individuals, however, is based on more than the difference in the sum of their tissue-specific proteins, or other antigenic molecules. It rests more broadly on the existence in each individual (or in all individuals of identical genotype, such as identical twins and members of a highly inbred strain) of specific molecules not restricted to certain tissues but common to all, or most, of its parts. (A third, but less secure, assumption states that similarly there are antigens common to all members of a species, the species-specific antigens.)

In recent reports of the Department, emphasis has been placed on studies of tissue specificity. During the past year important progress was recorded in

analyzing the chemistry and patterns of synthesis of the contractile proteins, including actomyosin and tropomyosin, of hemoglobin, and of the retinal photopigments. But what of the recognition mechanisms whereby an embryo develops the ability to distinguish isoantigens and tissue-specific antigens, and to make antibodies to them?

The year has been marked by several promising findings in this area. We know that exposure of embryos late in their development, and of newborn animals, to living adult cells induces a state of tolerance, for when these animals are challenged with living homologous cells in later life they are incapable of producing an immune reaction. The mechanism for producing an immune reaction to homografts develops only in the late embryonic stages, and during this period it is subject to modification. But what of the mechanisms for the production of antibodies to purified, nonliving, tissue-specific antigens? If tolerance can also be acquired to, say, the antigens of spleen, liver, or heart, the embryologist has a powerful tool, for, by paralyzing the immune mechanisms for a number of "common" antigens, he should be able to enhance the specificity of antiorgan sera of his choice. During the year Charles Wytenbach has made substantial progress toward this end. Although the results of his first large-scale experiments must be considered tentative, pending amplification and verification, it appears that tolerance of a high order is produced by the injection of newborn rabbits with antigens of liver and spleen. These promising experiments are being continued.

When embryos are exposed to living grafts of adult tissues, which themselves have the capacity to produce antibodies, an entirely different picture emerges. Last year the consequences of the reaction of grafts of adult spleen and lymphoid cells against animals experimentally deprived of the ability to produce antibodies were described—the so-called graft-versus-host reaction. The results were clouded by the inability to establish that the host's immune mechanisms had not recovered from the treatment. Ebert and his co-worker Louis E. DeLanney believe that they are now well on the way toward providing the critical evidence required. Experiments have been carried out, using both chick and salamander embryos, in which grafts of adult spleen were made before the host's antibody-forming tissues had begun to develop. Well in advance of the critical period for the onset of immune reactions the embryo is killed as a result of a widespread destruction of the vascular bed. This critical program was initiated during DeLanney's tenure as a Fellow of the Carnegie Institution of Washington. Continuing long-range cooperation between the Department and DeLanney at Wabash College is most gratifying.

Another significant contribution to the Department's program was made during the year by a visiting investigator from New Zealand's University of Otago School of Medicine, William E. Adams, who utilized the Bluntschli Collection in a rewarding study of the development of the adrenal gland and the

sympathetic paraganglia in insectivores. Especially noteworthy is his observation that the paraganglionic tissue is not confined to the adrenal region but extends alongside the sympathetic chain as a distinctive column, reaching to the base of the skull.

Several new investigations, begun during the year, have proved to be rewarding. Using the large native silkworm moth, *Hyalophora cecropia*, as experimental material, Hans Laufer has made a study of factors regulating protein synthesis in development. Employing immunochemical techniques, he has shown that certain antigenic constituents of the blood of the insect are regenerated after experimental bleeding. The regenerated antigens are proteins—and he has been able to demonstrate, through an ingenious combination of immunochemical and histochemical techniques, that at least one of them has enzymatic activity. It is of unusual interest that an enzyme can be identified histochemically while in combination with its antibodies. Suggestive studies of this kind pose, and attempt in some part to answer, the question why some kinds of molecules are replaced, but not others. Their primary purpose is not to make a chemical inventory of the proteins and their changes in development (although this is an obvious first step if there is to be subsequent progress) but to gain further insight into the reasons for the changes.

The final level of biological organization, that of many-celled individuals into species and populations, is of particular interest to the Department of Plant Biology, whose work in experimental taxonomy, extending over many years, has become classic. This year has been notable for these programs. There has been what almost amounts to the initiation of a new field of investigation. The year has also seen the maturation and completion of work in an older one.

For many years the program of experimental taxonomy has been concerned with the manner in which populations of plants become adjusted to many environments (living as well as nonliving), viewing the changes they undergo as processes of genetic transformation, reflected in morphological and to a degree in physiological change. The emphasis in these studies of plant relationships has progressed in recent years from primarily cytogenetic and transplant investigations to those in the area of comparative physiology. Why some plants thrive in a particular environment while other, closely related forms require a very different climate, even for survival, has become one of the main questions under study.

Different species and strains of the monkey flower *Mimulus* have been found particularly suitable for comparison of physiological behavior as measured in the laboratory. Not only are they able to grow in the contrasting environments of the three experimental gardens of the Department at Palo Alto, Mather, and Timberline, but their size and adaptability make them excellent subjects for laboratory investigation. A number of climatic races of *Mimulus* plants are being grown at the three altitude stations as material for laboratory studies

of the way in which their rates of photosynthesis and respiration vary in response to changes of temperature and light intensity. Recorded patterns of photosynthesis and respiration from these climatic races, taken under controlled conditions of light and temperature, will be compared with the observed morphological growth responses of the same plants at the different altitudes and in experimental growth chambers in which temperature, light, and humidity can be accurately controlled.

The rates of photosynthesis and respiration of clones of *Mimulus* taken originally from diverse latitudes and altitudes have been explored in a preliminary survey. The results obtained to date indicate marked differences among them. The interpretation of these differences in terms of climatic races and their significance, if any, in natural selection in different environments is an ultimate objective of the investigations.

In segregating progenies of the cross of two species of *Mimulus* taken from different altitudes (*M. cardinalis* and *M. lewisii*) the frequencies of certain flower colors were found to vary greatly with the altitude at which the progenies had been established. At high elevations types resembling the alpine parent were predominant, while types resembling the lowland parent were favored in the milder climates. There appears to be a genetic linkage between floral characters and the physiological characters that determine survival under extreme conditions.

Studies have been begun of the germination of seedling populations of contrasting parental and hybrid lines of *Mimulus* under crossed gradients of two controlled variables—temperature and light intensity. This technique is also being used to compare differences in germination capacity in races from contrasting habitats and in their segregating F_2 progeny. Individuals among the segregates which appear to differ in their temperature and light requirements may be selected and subjected to further field and laboratory tests.

This year marks the completion in the Department of the range-grass program described in 1954, involving a series of breeding and testing experiments undertaken in cooperation with the Agricultural Research Service of the Department of Agriculture. They were designed to test the responses of apomictic strains of key parental and hybrid bluegrasses in widely divergent climates at various experiment stations strategically located throughout the United States. The results illustrate to a striking degree the climatic specificity of the regional responses of the apomictic strains. This is the first time that such a series of widely distinct parental species and their stabilized apomictic hybrid derivatives have been systematically studied over a great range of climates on what amounts to a nation-wide basis. These tests therefore are of general biological as well as of more specific agronomic interest. The extraordinary wealth of information accumulated in the Department about the grasses of the genus *Poa* is being analyzed and summarized preparatory to publication.

LOSSES . . .

The death of Ernest Orlando Lawrence and of Howard E. Tatel during the year brought losses to the Institution which can never be repaired. Dr. Lawrence had served as a Trustee of the Institution for fourteen years. Dr. Tatel had conducted distinguished research in geophysics and seismology in the Department of Terrestrial Magnetism, where he was chairman of the earth physics section, for the past ten years.

Dr. Lawrence was born in Canton, South Dakota, on August 8, 1901. He attended the University of South Dakota, from which he was graduated in 1922, taking his master's degree at the University of Minnesota and his doctorate at Yale, where he became an assistant professor. In 1928 he moved to the University of California.

It was in these years that Lawrence conceived the idea of the cyclotron, which can be regarded as having initiated the modern era of experimental investigation in nuclear physics and for which—together with the great program of research that accompanied the development and was made possible by it—he was awarded the Nobel prize in 1939. In 1936 he established the Radiation Laboratory at the University of California, where his research was continued and of which he served as Director for the remainder of his life. That Laboratory created and continues to occupy a unique position in the whole fabric of American research in the physical sciences.

During World War II and thereafter Dr. Lawrence rendered services to the nation that can perhaps never be adequately estimated, especially in the fields of atomic development, of national self-defense, and of international relations. Death came at the close of a particularly arduous period of public service, as a representative of the United States at the international conference on scientific detection of nuclear explosions held at Geneva during the current summer.

Dr. Tatel died on November 15, 1957, in Washington, D. C. He had just returned from the seismic expedition of the Department of Terrestrial Magnetism to the high Andes. He was born in New York City on December 22, 1913. He attended the Massachusetts Institute of Technology, where he took both his undergraduate and his master's degrees, and did his doctoral work at Stanford University. He spent the following two years as a research associate in nuclear physics at the University of Michigan.

During World War II Dr. Tatel was engaged in research and development work at the Applied Physics Laboratory of the Johns Hopkins University, where he pioneered in the development of the proximity fuse and in the early developmental phase of ram jet propulsion. In 1947 he came to the Department of Terrestrial Magnetism, where his research in seismology and geophysics was of great importance. Especially noteworthy were his field work with colleagues of the Institution on the thickness of the earth's crust, and his laboratory model

work, which provided illuminating analyses of seismic phenomena. In recent years he devoted much attention to the development of special equipment for radio astronomy and to measuring the hydrogen clouds of our Galaxy. Dr. Tatel will be long remembered by his associates, not only for his high professional accomplishments, but even more for the quality which his personality brought to everyone associated with him and to every enterprise in which he was engaged.

Dr. Walter Baade, astronomer at the Mount Wilson and Palomar Observatories, retired on June 30, 1958, completing a career of research embodying contributions to astronomy of the highest order.

On coming to the Mount Wilson Observatory in 1931, Dr. Baade first attacked a series of photometric problems, including the establishment of photometric standards in Selected Areas, investigations of the light-curves of supernovae, and studies of the magnitudes of variables in globular clusters and in galaxies and their use for distance determinations. During World War II he took advantage of the darker skies consequent on the black-out in the Los Angeles area and of the development of faster red-sensitive plates to undertake those critical galactic investigations that led to the formulation of the concept of Population I and Population II stars discussed elsewhere in this report; with colleagues he made detailed studies of the color-magnitude relationships in representative samples of the two populations. The results of these investigations provided the observational basis for present theories of stellar evolution.

With the completion of the 200-inch Hale telescope Baade continued his studies of the Andromeda galaxy and the other members of the local group, paying special attention to cepheid variables and other stellar types that might be used as indicators for fixing the distances of these objects. This work represented the first step in the precise determination of the distances of all objects outside the Milky Way. The observations provided much of the evidence for a revision of the absolute magnitudes of the cepheid variables and a resultant increase in the distance scale of all objects outside our Galaxy by a factor of nearly 3.

In collaborative work with Dr. Minkowski, Dr. Baade also identified many of the celestial radio sources with optically observed objects and contributed much to the physical interpretation of the nature of these sources. From 1953 until his retirement, Dr. Baade served on the Observatory Committee of the Mount Wilson and Palomar Observatories.

. . . AND GAINS

The fellowship program in the natural sciences, made possible by a generous gift from the Carnegie Corporation of New York and described in last year's report, has brought to the Institution a group of distinguished investigators.

During the year fellowships were awarded to Dr. Hessel de Vries of the University of Leiden, Dr. David G. Catcheside of the University of Birmingham, Dr. Mogens Westergaard of the University of Copenhagen, and Dr. Evelyn E. B. Smith of the University of Glasgow.

It is a special pleasure to report that Dr. Vannevar Bush, retired President of the Institution, received the New England award of the Engineering Societies of New England on November 12, 1957. The award is presented annually to an engineer in New England who "merits recognition of his accomplished work as well as his character."

Dr. George W. Corner, the former Director of the Department of Embryology, received the Passano Foundation award of the American Medical Association at its convention in San Francisco on June 25, 1958. The award was made in recognition of "his long and continuing researches and for many fruitful contributions to the better understanding of mammalian anatomy and physiology, with particular emphasis on human reproduction." Both the University of Chicago and the Woman's Medical College of Philadelphia conferred honorary degrees on Dr. Corner during 1958.

It gives me much pleasure to announce the following honors that have been received during the year by the members of the staff of the Institution:

Dr. Horace W. Babcock, the assistant director of the Mount Wilson and Palomar Observatories, was presented with the Henry Draper medal of the National Academy of Sciences on April 28, 1958, for his "original and outstanding work leading to the discovery of magnetic fields in stars and also the general magnetic field of the sun." He was also awarded the Eddington medal of the Royal Astronomical Society for his research on the magnetic fields of early-type stars and of the sun.

Dr. Allan Sandage, astronomer at the Observatories, received the Helen Warner prize, given by the American Astronomical Society for outstanding research by younger members of the Society. The prize was given especially for his investigations of the extragalactic distance scale.

Dr. Hatten S. Yoder, petrologist at the Geophysical Laboratory, and Dr. Alfred D. Hershey, microbiologist at the Department of Genetics, were elected to membership in the National Academy of Sciences on April 29, 1958.

Dr. Barbara McClintock, cytogeneticist at the Department of Genetics, received the degree of Doctor of Science, *honoris causa*, from Smith College on June 8, 1958. She also received on August 28, 1957, a Certificate of Merit from the Botanical Society of America "in recognition of distinguished achievement in and contributions to the advancement of botanical science."

Caryl P. Haskins

REPORTS OF DEPARTMENTS

and SPECIAL STUDIES

MOUNT WILSON AND PALOMAR OBSERVATORIES

COMMITTEE ON IMAGE TUBES FOR TELESCOPES

DEPARTMENT OF TERRESTRIAL MAGNETISM

GEOPHYSICAL LABORATORY

DEPARTMENT OF PLANT BIOLOGY

DEPARTMENT OF EMBRYOLOGY

DEPARTMENT OF GENETICS

DEPARTMENT OF ARCHAEOLOGY

MOUNT WILSON & PALOMAR OBSERVATORIES

*Operated by Carnegie Institution of Washington
and the California Institute of Technology*

Pasadena, California

IRA S. BOWEN, *Director*
HORACE W. BABCOCK, *Assistant Director*

OBSERVATORY COMMITTEE

Ira S. Bowen, *Chairman*
Walter Baade ¹
Horace W. Babcock

Robert S. Bacher
Jesse L. Greenstein
Earnest C. Watson

¹ Replaced by Rudolph Minkowski, July 1, 1958.

CONTENTS

	<i>page</i>		<i>page</i>
<i>Introduction</i>	51	Expanding shells around novae and supernovae	66
<i>Observing Conditions</i>	56	<i>Globular and Galactic Clusters</i>	67
<i>Solar Observations</i>	56	White dwarfs in M 67.....	70
Solar photography	56	Absolute magnitudes of cepheids.....	70
Sunspot activity	56	Dynamics of clusters.....	70
Magnetic polarities	57	<i>Galaxies</i>	71
Solar magnetic fields.....	57	The local group.....	71
Corpuscular radiation	58	Rotation and dispersion of stellar ve- locities	72
<i>Planets</i>	58	Magnitudes and energy curves of gal- axies	72
Rotation of Venus.....	58	Distance scale	73
<i>Comets</i>	59	Velocities of galaxies.....	74
<i>Stellar Spectroscopy</i>	59	Supernovae	74
Chemical composition of stellar at- mospheres	59	Density of gas clouds.....	74
Carbon stars	60	Catalogue of galaxies and cluster of galaxies	75
Blue stars in the galactic halo.....	60	Multiple galaxies	75
Central star of the Crab Nebula.....	60	Spectra of intergalactic luminous mat- ter	75
Line blanketing	61	<i>Radio Sources</i>	76
Variable stars	61	<i>Theoretical Studies</i>	77
Magnetic stars	62	<i>Instrumentation</i>	77
Mass loss from late-type giants.....	62	<i>Guest Investigators</i>	79
The relationship of H and K emission to absolute magnitude.....	64	<i>Staff and Organization</i>	83
Radial velocities	64	<i>Bibliography</i>	85
<i>Gaseous Nebulae</i>	64		
Internal motions	64		
Radial velocities	65		
Densities of nebulae.....	66		

INTRODUCTION

The Hale 200-inch telescope on Palomar Mountain was dedicated on June 3, 1948, and the agreement for the joint operation of the Mount Wilson and Palomar Observatories became effective on April 1, 1948. As the tenth anniversaries of both these events fall in the present report year, it is appropriate to review briefly the observational programs carried out during this first decade of joint operation.

Between the two World Wars Hubble, using the 100-inch on Mount Wilson, was able to make preliminary measurements of the distances, diameters, and luminosities of the galaxies and thereby to provide definite evidence that the galaxies are huge systems of stars similar to our Milky Way, and the major units in which the mass of the universe is distributed. One of the major projects planned for the Hale telescope was a thorough restudy of the near-by galaxies, in particular a re-examination of the procedure for fixing their distances in order to eliminate the very large uncertainties that were known to exist in these initial measurements. In general this procedure consists of the comparison of the apparent brightness of some distance indicator, such as a star, in the galaxy with its absolute brightness or luminosity as determined from near-by examples in our own Galaxy. The inverse-square law for the falling off of apparent brightness with distance then gives the required answer. For use as such a distance indicator, the star or other object must be unusually bright so that it can be observed at large distances, and it must have some characteristic by which it can be identified as a particular type of star or object having the same absolute brightness as the examples measured in our own system. In his original measurements Hubble used cepheid variables as the chief distance indicator, since they could be identified by their light-fluctuations.

In 1952 Baade, from observations with

the 200-inch, Thackeray and Wesselink, from observations of the Magellanic Clouds at Pretoria, and Mineur as well as Blaauw and H. R. Morgan, from a theoretical study of proper motions of cepheids in our own Galaxy, independently arrived at the conclusion that the classical cepheids are about 1.5 magnitudes brighter than the value assumed in the earlier studies. A careful remeasurement was also made of the apparent magnitudes of a substantial number of cepheids in several of the galaxies close enough for their observation. Since the absolute luminosity of a cepheid variable is a function of its period, it is necessary to accumulate several dozen plates of each field, distributed over a sufficient length of time to give a reliable light-curve of each variable. For this purpose Baade obtained sets of plates for four fields in the Andromeda galaxy and for several other galaxies in the local group, including NGC 185, NGC 205, and the dwarf galaxies in Draco, Ursa Minor, and Leo I and II.

At the same time steps were taken to investigate the properties of other unusually bright objects that might be used as distance indicators to check the distances found from the cepheids and, if possible, to extend the measures to more distant galaxies. Hubble and Sandage measured the brightest stars in the Andromeda galaxy, including the very bright irregular variables. Sandage investigated the integrated magnitude of the gaseous nebulae or H II regions surrounding several of these very luminous stars. Code and Dr. T. E. Houck have compared the exceptionally bright blue stars in NGC 6822, M 33, the Large Magellanic Cloud, and our own Galaxy. Arp made a very extensive study of the magnitudes, light-curves, and frequency of occurrence of ordinary novae in the Andromeda galaxy. For this purpose fields in the galaxy were photographed with the 60-inch on every

clear moonless night during two observing seasons. A total of thirty novae was found during this period. Measurements of the distribution in magnitude of the globular clusters in the Andromeda galaxy were made by Baum. Humason and Sandage made observations of the red supergiants in M 33. Hubble, Sandage, and Baade observed various distance indicators in the more distant galaxies outside the local group, including M 51, members of the M 81 and M 101 groups of galaxies, and the galaxies in the Ursa Major cloud and the Virgo cluster.

Finally, it was necessary to recalibrate the magnitudes of the stars in Selected Areas that had been used as standards of magnitude for the very faint stars in these galaxies. This recalibration with photoelectric techniques was started by Drs. J. Stebbins and A. E. Whitford of the Washburn Observatory using the 100-inch telescope, and was completed by Baum with the 200-inch. These observers found that the earlier photographically determined magnitudes of very faint stars were too bright by as much as 1 magnitude.

When these corrections are made, the Andromeda galaxy turns out to be nearly 3 times as distant as was indicated by the earlier measurements. Likewise, the diameter and mass of this galaxy must be increased by a similar factor, and the luminosity by a factor of about 8. The application of the new values of the magnitudes of various distance indicators to the preliminary observations of the more distant galaxies points to an even larger correction to the distances and dimensions of these objects. Thus Sandage estimates that the old distance of the Virgo cluster and all more distant galaxies should be increased by a factor of between 5 and 10. If further measurements confirm this estimate, the size of the observable universe must be increased by the same factor.

During World War II Baade had observed with the 100-inch that the most conspicuous stars in the nucleus of the Andromeda galaxy and in the elliptical

galaxies are red giants whereas in the spiral arms of the Andromeda galaxy and in the neighborhood of the sun in our own Galaxy the brightest stars are blue. This observation led to the concept of population types, the stars in our own neighborhood being designated as Population I and those in the galactic nuclei and in the elliptical galaxies as Population II. This concept raised many new questions as to the cause of the different characteristics of the two population types. It also introduced many new uncertainties into the distance-scale problem by questioning the validity of the assumption that the magnitude of a given stellar type used as a distance indicator is the same in all objects. This last point was especially serious, since in many of the older measurements the absolute magnitude was determined from examples in one population and the apparent magnitude in a distant object from examples of the other population.

An extensive study of the properties of stars of the two population types was therefore instituted. Measurements of the color-magnitude relationships were made for various groups of stars, including several globular clusters and Galactic clusters, observed by Arp, Baum, Osterbrock, Sandage, Schmidt, and Walker, and a few members of the local group of galaxies investigated by Baade and Sandage. Studies were also made of the period-luminosity function of cepheid and cluster-type variables in these objects.

From these studies the picture soon emerged that Population I is characterized by very young stars that have only recently condensed from clouds of dust and gas. This assumption was confirmed by the observation that stars with Population I characteristics are invariably associated with such gas and dust clouds. On the other hand Population II contains only old stars. Further confirmation of this picture was obtained from parallel theoretical and laboratory developments which showed that the energy radiated by the stars comes

from the transformation of hydrogen into helium and the heavier elements in the hot stellar core. The detailed analysis of the nuclear processes occurring in these stars was developed by Hoyle and G. R. Burbidge in collaboration with Drs. W. A. Fowler and E. M. Burbidge of the Kellogg Radiation Laboratory. The effect of the depletion of the hydrogen fuel on the characteristics of the star was investigated theoretically by Hoyle and by Dr. Martin Schwarzschild and others.

In general, when a mass of predominantly hydrogen gas condenses into a star on the main sequence, the luminosity and surface temperature on this sequence are fixed by the mass condensed: the luminosity varies approximately as the cube of the mass, and the surface temperature increases slowly but continuously with the mass. Because of the rapid expenditure of the hydrogen fuel by the very massive stars their fuel soon becomes exhausted. As depletion of the fuel progresses the star expands and its surface cools while the total radiation increases; that is, the star leaves the main sequence at the critical stage of fuel depletion. When the fuel is finally exhausted the radiation rapidly falls off and the star probably ends its career as a faint white dwarf.

On this picture a young group of stars is characterized by a continuous progression of properties from the massive, extremely luminous, blue stars to the reddish faint dwarf stars. As the group becomes older the very luminous blue stars use up their fuel and drop out, and the brightest stars in such a group are the stars of moderate initial luminosity which because of approaching depletion of fuel have expanded and cooled off but have become temporarily more luminous. Such a stellar group, a few billion years old, should have the distribution in luminosity and color found to exist among the globular clusters and the elliptical galaxies.

The discovery of the role played by nuclear transformations in the energy production and the evolution of the stars gave

an added interest to spectroscopic studies of abundances of the elements participating in these transformations in stars of various ages. Fortunately the great light-gathering power of the 200-inch and the efficiency of its spectrographs made possible the study of the faint stars such as the globular-cluster stars and the white dwarfs which are of special significance in evolutionary theory.

Deutsch, Greenstein, and Olin Wilson carried out extensive observations of the old stars in several globular clusters that are typical representatives of Population II. In general these studies indicated that the metal content of the Population II stars is lower than that of the Population I stars, although the abundance of the metals in different clusters appears to range from nearly equality to that of Population I in some clusters down to less than 1 per cent of this in other clusters such as M 92. Similarly, although the color-luminosity curves of these clusters in general follow the pattern predicted by theory, small unexplained differences between different clusters of nearly the same age are also apparent.

Greenstein also made spectroscopic analyses of some dozens of the faint white dwarfs, which, as mentioned above, probably represent the final stage of evolution of a star. He found striking differences in their spectra and presumably in the composition of their surface atmospheres. Greenstein also investigated the differences in chemical composition between dwarfs, subgiants, and giant stars. The compositions of stars of high and low velocity were also compared, as there is evidence that they may be representatives of the two population types.

Deutsch has investigated the processes by which the massive stars, when in the expanded red-giant stage, eject into space an appreciable fraction of their mass. These processes provide a probable explanation for the large differences between the initial mass of the blue giant stars and their final mass in the white-dwarf stage.

At the same time they explain the presence of observable amounts of the heavy elements in the interstellar gas.

One phase of the study of galaxies initiated in the 1920's and 1930's with the 100-inch was the measurement of their radial velocities by Humason and the discovery by Hubble of the linear relationship between these velocities and the distances. On its completion Humason used the 200-inch telescope and its prime-focus spectrograph to measure the velocities of a substantial number of additional galaxies, and in particular to extend the measurements out to objects having a velocity of recession about 20 per cent that of light. At the same time Drs. J. Stebbins and A. E. Whitford of the Washburn Observatory and Pettit used the 60-inch and 100-inch instruments to measure photoelectrically the magnitudes of the galaxies whose velocities have been determined. In 1956 Humason, Sandage, and Dr. N. Mayall of the Lick Observatory published a list of the velocities of 600 galaxies measured at the Mount Wilson and Palomar Observatories and of 300 galaxies observed at the Lick Observatory. These authors then made a detailed analysis of the velocity-distance relationship based on the observed velocities and the distances as deduced from the magnitudes.

By means of eight-color photometry Baum has determined the shape of the curve of radiated energy versus wavelength for a number of galaxies at a wide range of distances and has measured the radial velocities from the shifts in these curves. Redshifts as great as $\Delta\lambda/\lambda=0.4$, well beyond the range of definite spectroscopic observations, have been found. Indeed it is now becoming evident that the primary factor that sets the limit on the distance to which a large telescope like the Hale 200-inch can observe a galaxy or cluster of galaxies is not the falling off of the light because of increasing distance but the redshift of light from the galaxy into the far infrared where photographic plates

are no longer sensitive and where atmospheric absorption is large and atmospheric "night-sky" radiation is strong.

Various properties of galaxies have been investigated. Thus Münch has made spectroscopic observations of M 81 to determine its rotation and mass. Oort and Minkowski have investigated the rotation, the mass distribution, and the range of random stellar velocities in NGC 3115. Multicolor measurements of the integrated light of a large number of globular clusters and galaxies have been made by Baum and Tifft for study of their stellar contents. Code has made similar studies of the energy distribution in galaxies with a spectroscopic scanner for the same purpose. Studies of the luminosity function and of the distribution and clustering of galaxies were carried out by Zwicky. Zwicky also investigated the faint blue stars and other objects in the halo about our own and other galaxies.

After the discovery of strong radio sources in space, Baade and Minkowski were able to identify several of the strongest of the sources with peculiar optically observed objects. Several sources, Cygnus A, NGC 1275, and probably NGC 5128, turn out to be pairs of galaxies in collision. It is thought that the strong radio waves emitted have their origin in the collision of the gas clouds present in the spiral arms of these pairs of galaxies. The remains of two explosions of supernovae, the Crab Nebula (Supernova of A.D. 1054) and a faint nebula in Cassiopeia, first observed by Minkowski and identified by him as the remains of the Supernova of 1572, were shown to be identical with radio sources. The discovery in the USSR of the polarization of the light from the Crab Nebula followed by detailed studies of the polarization by Baade confirms the suggestion that much of the observed light from this object is synchrotron radiation from electrons of very high velocity accelerated in a small magnetic field. Similarly, the observations by Baade of the polarization of

the light from the jet of the Galaxy M 87 which is also a radio source points to synchrotron radiation as the origin of much of the light from this object.

The discovery by H. W. Babcock in 1946 of a star having a general magnetic field with a strength of several thousand gauss has been followed by a survey of many stars of similar types. Of 305 stars examined, 84 show a definite magnetic field and an additional 55 probably have such a field. Since in many stars the magnetic fields fluctuate more or less regularly with a period of a few days, it has been necessary to make extensive observations of many of these objects over a long period of time. The spectroscopic and other properties of these magnetic stars have been studied by Deutsch in an effort to build up a model to explain their behavior.

With the development of the solar magnetograph by H. D. and H. W. Babcock it has been possible to map the magnetic fields over the surface of the sun with an accuracy of a few tenths of a gauss. Daily maps over a period of a few years provide definite evidence for persistent polar fields and for fluctuating fields over the equatorial regions of the sun. The observed fluctuations explain why early attempts by Hale and others to measure a general field of the sun led to such inconclusive results. Sunspots, prominences, flares, and other solar phenomena have been investigated by Nicholson and Richardson on photographs and spectroheliograms taken daily at the solar towers on Mount Wilson.

Extensive studies have been made of the gas clouds in our own Galaxy. Minkowski has carried out surveys that have nearly doubled the number of known planetary nebulae. Olin Wilson has developed spectroscopic techniques that have enabled him to study the internal motions of the planetary nebulae and, with Münch, to map the motions of the gases over a large part of the brighter areas of the Orion nebula. Osterbrock has used a new technique to measure densities of the gas in various nebulae.

During the decade Ralph Wilson completed the radial-velocity program of the Observatories. This program included the measurement of several thousand plates, many of which were accumulated during World War II, and the publication of the radial velocities of over 2400 stars. A compilation listing the positions, magnitudes, spectral types, and definitive radial velocities of all the 15,105 stars whose velocities have been determined at any observatory was then published by Wilson with the title *General Catalogue of Stellar Radial Velocities*.

Olin Wilson with the assistance of Bappu made a detailed study of the widths of the emission components at the center of the H and K lines of stars later than G0. Wilson found a linear relationship between the logarithm of this width and the absolute magnitude of the star that should prove a powerful tool for the determination of the luminosities, and therefore of the distances, of stars of these types.

A large number of observations were made by Merrill, Joy, and Sanford of the spectra of variable stars and of stars with prominent emission lines.

In a project sponsored by the National Geographic Society the 48-inch schmidt camera was used during most of the decade to map the whole sky north of declination -27° . Two photographs were taken, one in blue light and the other in red light, of each of 879 fields. Stars were recorded to the 21st magnitude on the blue plates and to the 20th magnitude on the red plates. This mapping represents a penetration to a distance about three times farther than previous surveys, or a coverage of about twenty-five times the volume of space. Over 120 copies of this National Geographic Society-Palomar Observatory Sky Survey in the form of 1758 photographic prints have been ordered and are being distributed to observatories throughout the world. From a search of the survey plates Abell compiled a list of 2712 clusters of galaxies, of which only a few dozen were known before.

Just before and during the observations for the Survey two very unusual asteroids, named Icarus and Geographos, that may come unusually close to the earth, were discovered with the 48-inch schmidt. In 1951 Nicholson discovered the twelfth satellite of Jupiter on plates taken with the 100-inch telescope.

The above describes the major programs that have been carried out by the permanent staff of the Observatories, the Carnegie Fellows and research fellows who spent one or two years each at the Observatories, and a number of graduate students from the Department of Astronomy at the California Institute. Because of the limited size of the staff, the programs have not required all the available time of the telescopes. Shortly before the start of joint operation of the two Observatories a guest-investigator program was inaugurated by which such facilities of the Observatories as were not needed by its own staff were made available to astronomers of other institutions. This program has been carried out on a cooperative basis, the visiting astronomers' own institution providing the

necessary leave and travel expenses and the Observatories furnishing the telescope time and the photographic plates and other supplies.

During the past decade 60 astronomers from 23 institutions in the United States made over 160 visits to the Observatories to carry out observations with the telescopes or in a very few cases to study plates already available in the files. In this same period 20 astronomers from 18 institutions in 12 other countries made 26 visits.

The optical tests of the Hale telescope reported early in the decade showed that technically the instrument performs fully as well as had been planned. The real test of the soundness of design of an instrument, its optical perfection, and the efficiency of its auxiliary equipment comes from its ability to solve astronomical problems. The foregoing record of the first decade of operation of the 200-inch telescope and its supporting instruments on Palomar Mountain and Mount Wilson provides the final answer as to the success of Hale's dreams and hopes for the telescope that now bears his name.

OBSERVING CONDITIONS

For the second time in the past eleven years precipitation was above normal on Mount Wilson, with a rainfall of 56.99 inches. Because of the large number of cloudy days observing conditions were

poor. Solar observations were made on 327 days, and observations were made on 292 nights with the 100-inch telescope and on 272 nights with the 60-inch telescope.

SOLAR OBSERVATIONS

Solar Photography

Solar observations were made by Cragg, Hickox, Nicholson, Richardson, and Seyfert. The numbers of photographs of various kinds taken between July 1, 1957, and June 30, 1958, were as follows:

Direct photographs	648
H α spectroheliograms, 60-foot focus . .	543
H α spectroheliograms, 18-foot focus . .	1,200
K2 spectroheliograms, 18-foot focus . .	909
K2 spectroheliograms, 7-foot focus . . .	55,600
K prominences, 18-foot focus	1,008

Sunspot Activity

The magnetic classification and study of sunspots and related phenomena have been continued by Nicholson and Cragg. Cooperative programs have been carried out with the U. S. Naval Observatory, the University of Michigan, the Observatory of Kodaikanal, the Meudon Observatory, the Central Radio Propagation Laboratory, and the Naval Research Laboratory. During the calendar year 1957, solar observations were made on 310 days, on none of

which was the sun without spots. The total number of spot groups observed in 1957 was 855, compared with 642 in 1956 and 208 in 1955. Previous to 1957 the largest number of groups observed here in one year was 663 in 1947. The largest number of groups ever observed here on one day was 27 on December 31, 1957. Previous to this cycle the highest average number of sunspot groups per month was 16.8 in May 1947. This number was exceeded in four consecutive months of this cycle, October, November, and December 1957, and January 1958. The current maximum is unquestionably the highest in recorded sunspot history.

The number of sunspot groups in high latitudes has been exceptionally large in recent years. In the 65 years from 1878 to 1943 only four groups were seen on more than one day farther than 40 degrees from the equator. In the 10 years from 1943 to 1953, eight such groups were observed, and in the present cycle twenty-seven have already been observed, four in 1955, seven in 1956, eleven in 1957, and five in the first half of 1958.

A large, active prominence that erupted on June 15, 1958, was photographed on June 12, 13, 14, and 15. On June 15 during the eruption 72 exposures were made. The prominence reached a height of 1.7 solar radii (735,000 miles) and a terminal (maximum) velocity of 175 miles per second.

The monthly means of the number of groups observed daily for the past two and one-half years are shown in table 1.

Magnetic Polarities

Magnetic polarities in each spot group have, if possible, been measured at least once. The classification of groups observed between July 1, 1957, and June 30, 1958, is indicated in table 2. "Regular" groups in the northern hemisphere are those in which the preceding members have N (north-seeking) polarity; in the southern hemisphere the polarities are reversed.

Solar Magnetic Fields

Observations with the solar magnetograph have been continued by Harold D. Babcock at the Hale Solar Laboratory on nearly all clear days. Much attention has been paid to the weak, high-latitude magnetic fields of the sun, and the sequence of observations accumulated since 1952

TABLE 1

Month	Daily Number of Sunspot Groups		
	1956	1957	1958
January	6.4	12.7	17.9
February	9.3	10.9	14.3
March	10.6	13.6	13.2
April	10.8	14.0	16.0
May	9.9	12.7	15.8
June	9.3	15.1	14.0
July	9.3	14.1	...
August	13.5	12.4	...
September	15.2	13.8	...
October	12.9	18.9	...
November	11.8	16.9	...
December	13.8	19.7	...
Yearly mean ..	10.2	14.6	...

TABLE 2

Hemisphere	Regular	Irregular	Unclassified
North	323	9	144
South	267	7	149
Whole sun..	590	16	293

shows that systematic changes, undoubtedly related to the progress of the main solar cycle, have taken place.

Howard employed the solar magnetograph which is installed at the 150-foot tower to investigate magnetic fields and Doppler shifts on the surface of the sun with good definition. An entrance aperture 10 seconds of arc square is normally used. From some tracings made in October it was discovered that the photospheric magnetic field in active regions, which is of the order of 30 gauss, takes very nearly the form of the calcium plage

regions. Observations were made later to determine whether deviations of the magnetic field from the exact shape of the plage regions, which sometimes occur, may be due to the fact that the magnetograph measures only the component of the magnetic field in the line of sight and that all the lines of force may not be parallel. A number of regions near sunspots have been traced, and it is hoped that the regions where the lines of force of the sunspot fields re-enter the photosphere can be located.

A number of traces of the magnetic-field strength along the sun's equator made in October showed that magnetic features of the order of 5 gauss exhibit some changes over a period of a few hours. Whether these changes represent real growth and decay of the field, or whether the fields moved slowly out of the path of the traces, could not be determined. More observations designed to differentiate between the two possibilities are planned.

A method was devised to measure magnetic fields and motions in sunspots. An aperture 5 seconds square is used. A number of measures were made of a spot as it crossed the solar disk. The object of this investigation is to determine whether or not the outward motion (Evershed effect) in sunspot penumbrae is parallel to the lines of force of the magnetic field. A device designed by Dr. Robert Leighton is being used in conjunction with the magnetograph to obtain an accurate determination of the differential rotation of the sun. The accuracy obtained for relative Doppler shifts in one day's observations is roughly 0.03 km/sec. Observations can be made clear to the poles.

Corpuscular Radiation

Wildey found, from a theoretical study of the transmission of solar corpuscular radiation (100 km/sec) in the interplanetary dust cloud, that the attenuation was virtually zero.

PLANETS

Rotation of Venus

Observations of Venus for rotation were made by Richardson in 1956-1957 at the Snow telescope with a coelostat and concave-mirror system, which produced an image of the planet on the slit from 1.6 to 3.9 mm in diameter, during eastern elongation. A grating with 900 lines per mm was used in a constant-temperature pit with a Littrow spectrograph of 18 feet focal length. This arrangement used in the 2nd order gave a dispersion of 0.84 Å/mm. With a slit width of 0.1 mm a satisfactory exposure could be obtained in 45 minutes, although more than an hour was desirable if time permitted. The emulsion was Eastman 103a-E(2) used with Corning filter 3486, which gives 80 per cent transmission longward of 5570 Å.

Observations when Venus was west of the sun were made at the coudé focus of the 100-inch telescope with the 114-inch camera. Measures on 102 lines from 8 of

the best plates were selected for discussion. The straight mean of the 102 measures gave a limb velocity of -0.032 km/sec, corresponding to a rotation period of 14 days, retrograde. The standard error of the mean is ± 0.033 km/sec.

Checks on the measures at the Snow telescope were obtained on the sun by using a short-focus lens that gave a solar image about the size of Venus. Observations for solar rotation were made from heliocentric latitude 3° to 71° . They agreed closely with the solar rotation in these latitudes obtained by Adams. Checks at the 100-inch were obtained from spectra of Mars which gave a rotation period of 24.2 hours, as compared with the known rotation of 24.6 hours.

That observations were not taken pole-on was checked from the inclination of the axis of Venus as determined by G. P. Kuiper and Richardson, on the assumption that the ultraviolet cloud belts are parallel to the planet's equator.

COMETS

A series of direct photographs of the bright comet Mrkos (1957d) was obtained with the 48-inch schmidt telescope by Kearns under the supervision of Osterbrock. The plates were taken for a physical study of the material in the tail, and they have been sent to Professor Biermann of the Max Planck Institut, who will supervise their measurement and reduction. A preliminary survey of the plates by Kearns resulted in the discovery of a feature which not only moved from the head of the comet away from the sun along the tail, but also appeared to move from one side of the tail to the other. This observation shows that the comet tail either rotated or oscillated, with a period of the order of four days.

Greenstein obtained spectra of Comet Mrkos (1957d) with the highest resolution ever employed for cometary spectra, namely, 18 Å/mm in the blue and 27 Å/mm in the red. The analysis of the $\lambda 3883$ CN band permitted a very detailed confirmation of the Swings fluorescence mechanism. In addition, small velocity differences along the radius vector of the sun shifted individual rotational lines sufficiently, with respect to the solar absorption-

line spectrum, to produce intensity changes between the side of the comet head facing the sun and the side away from it. The structure of the Na I lines showed a similar fore-and-aft asymmetry, being strong but sharply cut off on the side facing the sun, and streaming away more uniformly toward the tail. An atlas of the visual spectrum ($\lambda\lambda 4900-6800$) has been prepared, and about 400 lines, largely C_2 and NH_2 , have been measured. The red CN system does not appear. The identification of [O I], made in collaboration with Dr. P. Swings of the University of Liège, seems definite. Detailed analysis of these spectra will be carried out at Liège.

Periodic Comet Wolf I (1951 VI) was recovered on plates exposed by Baum on the night of June 12 at the prime focus of the 200-inch. This work was done in cooperation with Dr. Elizabeth Roemer of the U. S. Naval Observatory at Flagstaff, who supplied the information required for undertaking recovery and who measured the plates to obtain corrected positions. The visual magnitude of the comet as determined indirectly was 20.4 on the night of observation.

STELLAR SPECTROSCOPY

During the report year 828 spectrograms were made with the 200-inch telescope, 965 with the 100-inch, and 932 with the 60-inch.

Chemical Composition of Stellar Atmospheres

An extensive project on the chemical composition of stellar atmospheres is in progress under the supervision of Greenstein with the financial support of the Office of Scientific Research of the U. S. Air Force. One major investigation has been the determination of abundance differences between very old and very young stars. Spectra of the brightest red giants in M 92 and M 13 taken by Greenstein

have been compared with those of the field high-velocity star HDE 232078 and a young K3 II star in the Galactic cluster M 41. Helfer and Wallerstein find abundance deficiencies of the metals largest in M 92 (factor of 200), next in M 13 and HDE 232078 (factor of 20), and small or absent in the M 41 star. All metals seem to share about equally in this deficiency.

Two dwarf stars in the Hyades, a relatively young Galactic cluster, have been compared with the sun, and with the old, high-velocity star 85 Pegasi. This investigation is being carried out using relatively unblended lines in the yellow-red, with many Mount Wilson coude plates per star, to see whether any small abundance dif-

ferences of the metals can be detected, caused by a difference of at least 3.5 billion years in epoch of formation of the stars.

The peculiar red supergiant VY Canis Majoris has been observed by Wallerstein. The outer envelope of this star contains emission lines of sodium, calcium, and potassium (the last observed for the first time in any star).

The reddish white dwarf van Maanen 2 has been investigated very extensively by Weidemann. Models have been computed for values of the effective temperature 6600° , 5700° , and 5000° K, with $\log g = +7.5, 8.0, 8.4$. The profiles of the blended and broadened ultraviolet Fe I lines have been resolved into their components, taking into account the dependence on depth and the pressure broadening of the van der Waals type. The gas pressure is found to be about 1000 atmospheres, much higher than expected with normal abundances. Allowing the abundance of hydrogen ϵ_H and of the metals ϵ_M to vary, the observed damping constant and the central depths of the lines yield a decrease of $\log \epsilon_H = -1.6$, and of $\log \epsilon_M = -3.8$, with respect to the sun. The enormous decrease in metal abundances is hardly affected by allowing for the formation of molecules, although the hydrogen deficiency is reduced. The theoretical prediction of exhaustion of the hydrogen and low surface abundances of the metals is apparently confirmed. Much remains to be done in this field of phenomena at very high pressure.

Bonsack has made an extensive study of the neutral lithium lines in 47 G8 and M1 stars. About half the stars show Li I lines at 7 Å/mm. Curves of growth have been constructed, and the Li abundance has been determined. The search for another element important in theories of nucleogenesis, beryllium, has been abandoned because of the technical difficulties of observation at $\lambda 3130$.

An elaborate spectrophotometric study of the standard star, σ Bootis, F2 V, is being carried out by Greenstein. It is hoped

that this star, observed at Mount Wilson, Palomar, Victoria, and elsewhere, can be used to provide standards of equivalent width for other investigators.

Carbon Stars

A survey by Greenstein of high-dispersion spectra of the early carbon stars has revealed a number of differences in both the line and the band spectra. The high-velocity star HD 201626 seems to be of early type, related to the "barium" stars, and has very strong C_2 , CH, CN bands. Excellent spectra have been obtained of the peculiar carbon emission object, involved in a nebula, V348 Sagittarii. Some lines are complex in structure, double or of the Be or P Cygni type. The barium star ζ Capricorni has been found to show weak C_2 and C I lines.

Blue Stars in the Galactic Halo

Spectra of faint blue stars in the Galactic halo have been classified by Greenstein in an attempt to systematize the bewildering variety of objects found there. In addition to runaway Population I objects, many stars of Population II are found, including: (1) weak-line B and A stars, in which hydrogen lines are strong, deep, and sharp, but He I and Mg II are weak; these may be horizontal-branch stars; (2) subdwarfs, either of type O or, less easily recognizable, of type B, with relatively shallow lines. The search for new types of white dwarfs continues. The star LDS 749B is another member of the helium-rich type, DB. The object HZ 34 has an unusual and as yet unclassifiable spectrum.

Central Star of the Crab Nebula

Excellent spectra of the supposed central star of the Crab Nebula have been obtained by Zwicky with the 200-inch telescope prime-focus spectrograph. They are entirely continuous, no absorption or emission features being recognizable in them. Further attempts will, however, be made to record good spectra over the whole

available wavelength range, particularly in the ultraviolet, and to eliminate as much of the background light from the superposed nebulosity as possible by decreasing the width of the spectrograph slit.

Line Blanketing

Melbourne has nearly completed observations for a study of line blanketing in various types of stars. He used the photoelectric scanner to obtain monochromatic fluxes, and the coude spectrograph to determine the effects of the lines. The corrected fluxes are compared with predicted values from model stellar atmospheres. A total of 15 stars from O9 V to G4 V, F5 III to G8 III, and 2 subdwarfs are included in the program.

Variable Stars

In the hope of obtaining concurrent records of one of its frequent but unpredictable outbursts, continuous photoelectric observations of the light-changes of UV Ceti were made on four nights, October 4-7, 1957, by Paul Roques at the Griffith Observatory in Los Angeles with the 12-inch refractor, and on the same nights successive spectrograms were obtained with the 60-inch reflector on Mount Wilson by Joy.

No variations in brightness greater than 0.2 magnitude were recorded while the sky in Los Angeles permitted observations. On Mount Wilson, with excellent seeing and clear sky, 12 spectrograms of the fainter star of the pair (magnitudes 12.5, 13.00; separation $< 2''$; types dM5.5e, dM5.5e) were obtained with average exposures of 100 minutes. On October 7 during the third exposure the observer noted a sudden flare of more than a magnitude, and the star returned to normal brightness more slowly in about 10 minutes. Unfortunately, at this time the Los Angeles sky was covered with haze and fog. This spectrogram showed much wider and stronger emission hydrogen lines than the other plates of the series, and

the bright lines H and K of Ca II were somewhat strengthened. This observation confirms the results previously obtained for the same star at the time of the much greater flare of September 25, 1948.

On the same nights, three spectrograms each of V 371 Orionis and 20C1191 (types dM3e, dM3e) were obtained by Joy. Velocity variations were confirmed, but no certain changes in brightness or spectrum were noted.

Deutsch has the following variables under spectroscopic observation at the coude: VV Cephei, which is now in the egress phase of its chromospheric eclipse; RU Camelopardalis, a cepheid with carbon bands; ρ Cassiopeiae, which has many double lines; and R Cygni. An analysis by Merrill of the spectrum of this last star near maximum light has shown the presence of a great many narrow bright lines of metallic elements.

A brief summary of the work of the late R. F. Sanford on the complex and variable spectrum of AB Aurigae, type Aep, has been prepared by Merrill.

Tift has completed his photoelectric and spectroscopic investigation of a c-cluster-type variable, T Sextantis, and is reducing data on several additional cluster-type variables of classes a and c.

Wallerstein observed U, B, V colors of long-period variables with broad flat minima: U Cancr, Z Cassiopeiae, UX Cygni, Z Puppis, Z Tauri, RU Aurigae. Merrill has suggested that such flattened light-curves might be caused by faint companions. Wallerstein concludes, however, that these stars cannot have faint blue companions like Mira B; for Mira in the 1956 minimum the colors were clearly composite, $B-V = +1^m41$, $U-B = -0^m85$, while none of the above list showed any such ultraviolet excess.

Two symbiotic stars have been found by Greenstein at high Galactic latitudes, $+24^\circ2742$, $+37^\circ2318$. Both have P Cygni characteristics and a variable H α . The former shows strong Ca II emission, the

latter a composite, early-plus-late-type spectrum. The G star HD 117555, also at high latitude, has been extensively observed; discovered by Merrill to be rapidly rotating, it has very broad H α emission which varies in intensity and profile from night to night.

Wildevy has made a preliminary study of the bright variable in NGC 7006, and its location in the color-magnitude diagram.

Magnetic Stars

The catalogue of magnetic stars reported last year has been followed by a discussion by H. W. Babcock of the available data on magnetic phenomena among the stars of spectral type A. These stars have been classified into four groups according to the type of magnetic variation: the α group, showing cyclically varying fields of large amplitude with nearly symmetrical reversals of polarity; the β group, with irregular variations and seemingly random reversals of polarity; and the γ group, showing fluctuations of magnetic intensity but always the same polarity; in addition, a new (δ) group of magnetic stars that are also irregular spectrum variables has been identified. Ten of the 70 known magnetic A stars are spectroscopic binaries, of which 6 are newly identified. Only 1 of the α variables is a close binary, however, and in no others is the magnetic variation related to the binary motion. It is becoming increasingly clear that magnetic forces are dominant in governing the disposition of gaseous material in the vicinity of a star, and that these forces must be taken into account in the evolution of binary systems and in general cosmogonical problems.

The diverse phenomena observed in the magnetic stars invite further, more intensive investigation, and observations have been continued by Babcock on a regular basis with the 200-inch. Three stars that may be singled out as of particular interest are: (1) 53 Camelopardalis, an α variable with an 8-day period and a field varying

between the limits +3700 and -5100 gauss, the strongest yet observed in any star; (2) HD 32633, an α variable with a 4-day period and a decidedly irregular magnetic amplitude; and (3) HD 187474, unique in that its magnetic field has been invariant, and moderately strong, for over a year since its discovery.

Bonsack has analyzed the velocity variations of different lines in 56 Arietis. He found that the rigid, oblique, rotating model would satisfactorily explain the observed phenomena.

Among the peculiar A stars, the periodic magnetic spectrum variables HD 98088 and 53 Camelopardalis are under observation for harmonic analysis by Deutsch. Spectrograms of the ultra-sharp line "manganese star" Iota Coronae Borealis are being assessed for line identification at the Perkins Observatory.

Mass Loss from Late-Type Giants

Coudé spectrograms at dispersions of 10 A/mm, or brighter, are being accumulated by Deutsch to obtain evidence bearing on mass loss from late-type giants and supergiants. The observations at hand show that in different red giants the circumstellar components of strong resonance lines occur with widely different strengths. At 10 A/mm, the circumstellar spectrum can generally be recognized as such in stars that populate the Hertzsprung-Russell diagram above a line running from $M_v = -3$ at M0 to $M_v = 0$ at M5. In these objects, the H and K lines of Ca II show deep, rectangular absorption cores (H $_3$, K $_3$) superposed on emission features (H $_2$, K $_2$). The absorption cores of other resonance lines correlate well in strength with the strength of H $_3$ and K $_3$, indicating comparable ionization in all these circumstellar envelopes.

Circumstellar features are now recognized also in some K-type supergiants, and in a few long-period variables with types later than M5. A recent 10 A/mm spectrogram of χ Cygni at maximum brightness

shows conspicuous doubling of all strong resonance lines in absorption. Circumstellar features probably occur as well in the spectra of some N and S stars with effective temperatures and luminosities that are comparable with those of M giants and supergiants. Despite these correlations with type and luminosity, an appreciable dispersion exists in the circumstellar-line strength at any point in the Hertzsprung-Russell diagram.

Observations at 4.5 Å/mm of a few M giants of earlier type and lower luminosity show a composite structure at K_3 . This is typically a shallow absorption reversal in K_2 , with a deep but very weak core at the violet edge. The shallow absorption feature occurs alone in normal giants earlier than M0. In spectroscopic binaries, it moves with the lines from the reversing layer. It evidently arises not in a "detached" circumstellar envelope, but rather in the stellar chromosphere, at or near the levels responsible for K_2 . The deep, violet-displaced core represents the first detectable manifestation of the true circumstellar envelope.

A by-product of the coudé survey of late-type giants and supergiants is the discovery that many show a weak, wide emission line of Co I in the longward wing of K_1 , and some show an emission line of Fe II in the same region. These lines appear with much greater strength at certain phases in long-period variables. In the (relatively) nonvariable M stars they are probably related to the emission lines in the $\lambda 3200$ region, and they are evidence for hot and deep chromospheres in these stars.

For evidence on the dimensions of the circumstellar envelopes, the behavior of H_3 and K_3 is under study in several spectroscopic binaries containing late-type giants. In each of the following systems, H_3 and K_3 have been found to show stationary components that are not interstellar, or to yield a motion different from that of the reversing layer: ξ Cygni (K5 Ib),

RR Ursae Majoris (gM5), η Geminorum (gM3), 22 Vulpeculae (cG4), λ Andromedae (G7 III), and VV Cephei (M2+ Ia-Iab).

Visual binaries comprising late-type supergiants are also being surveyed by Deutsch for evidence of the α Herculis phenomenon, where the circumstellar envelope of the primary impresses its absorption lines on the spectrum of the visual companion. Antares probably shows the effect; the B3 V companion exhibits pairs of sharp absorption lines at H and K. The weaker pair is probably interstellar, the stronger circumstellar and due to the M supergiant. In the unique nebula found by Struve to surround the B star, sharp emission lines of Si II have been identified. These lines are evidence in support of O'Keefe's theory that quartz crystals condense in the gaseous ejecta from the M star, then volatilize again in the vicinity of the B star. The emission lines of [Fe II] reveal evidence of systematic motions superposed on the turbulence noted by Struve. The problem of the excitation of this nebula is still unsolved.

Mira is another visual binary that probably shows the α Herculis phenomenon at H and K. In this system, the hot companion lies near $M_v = +7$, in the same region of the Hertzsprung-Russell diagram as the explosive variables in the binary systems SS Cygni, AE Aquarii, and T Coronae Borealis. The observed photometric and spectroscopic instability of Mira B suggests that this star may be interacting with matter ejected from the late-type star, in a process analogous to that suggested by Kraft and Crawford for the other three systems. The possibility exists that all these explosive variables are really white dwarfs, with their excess luminosity deriving from accretion heating. There are obvious theoretical reasons for anticipating violent instabilities in a white dwarf subjected to accretion of hydrogen-rich material ejected from an aging red-giant companion. At the 1957 minimum of the long-period variable, Mira B was seen at

nearly the same position and brightness as at the preceding several minima.

The Relationship of H and K Emission to Absolute Magnitude

A total of 303 stars has been observed by Wilson for his investigation of the relation between the width of the H and K emission lines and the absolute magnitude. The great majority of these stars show H and K in emission.

Pending the possible derivation of a theoretical expression for the width-luminosity relationship which might conceivably by-pass the need for empirical calibration, one of the immediate needs is to improve the latter. For this purpose the four Hyades K-type stars have been extensively observed. When the accidental measuring errors are reduced in this fashion, it is found that the K-line widths place these four stars in the proper order of luminosity although the total range in their absolute magnitudes is only about 0.3 magnitude. Also the mean of measures of the Praesepe stars, less extensive than those of the Hyades, gives a distance modulus for Praesepe within 0.1 magnitude of that derived by the photometric observers. Thus, so far, the method appears to be capable of excellent accuracy. It is being used therefore to construct a color-magnitude diagram for late-type stars in the solar neighborhood.

A preliminary plot of this type, based on observation of stars with modern photoelectric colors which have been published in various lists, shows the following features: (1) The lower and right-hand edges of the distribution appear to be fairly sharply defined. (2) Except for a scattering of supergiants, most of the plotted points fall within the area bounded by the curves for M 67 and M 3. (3) The M 67

curve lies fairly close to the lower boundary of the distribution except near $B - V \sim 1.0$, where a small number of stars form a nearly vertical group well below the M 67 curve.

Confirmation of Wilson's photographic measurements of the half-widths of emission H and K in two stars was obtained by Code and Wilson using the photoelectric spectrum scanner described under instrumentation.

Radial Velocities

Over 140 radial-velocity spectrograms of a number of faint M dwarfs and a few standard stars were obtained by Woolley at a dispersion of 80 Å/mm with the 60-inch telescope. They were taken to fill certain gaps in the radial-velocity data necessary for a statistical study of the space velocity of stars within 20 parsecs of the sun.

An investigation of the radial velocities of distant stars in the Galactic region between longitude 340° and longitude 30° has been carried out by Luis and Guido Münch to provide data on the rotation of the inner parts of the Galactic system. The preliminary discussion of the material has shown that the observed motion of these stars is in good agreement with the rotational curve of the Galactic system proposed by M. Schmidt from 21-cm observations. In the course of this work it was found that the B0 Ia star HD 173438 is a single-line spectroscopic binary with a period around 240 days and a 90 km/sec range. The mass function of the system is then around $15 \odot$, and, for a mass ratio 2, the mass of the primary would be $200 \odot$. The O9 star HD 173783 has been found to be also a spectroscopic binary, but its period and range have not been determined with certainty.

GASEOUS NEBULAE

Internal Motions

Wilson has continued his investigation of the internal motions of nebulae using

the multislit technique, and a dispersion of 4.5 Å/mm at the 200-inch coudé. Most of the planetary nebulae, except for the ir-

regular ones, are more or less elliptical or ovoid in outline. One may ask whether the present shape of such objects is a manifestation of nonspherical velocity distribution at the time of ejection and, if so, whether such a velocity distribution can be revealed by observation.

Imagine two ellipsoidal nebulae, one of which is viewed at right angles to the major axis while the other is seen at an angle of 45° ; suppose that both are observed with a multislit, and that the central slit is set on the nucleus of each. Then it follows that for the first nebula the double line produced by the central slit is symmetrical, as are also the double lines on either side of it. The same is not true, however, for the nebula viewed at 45° . Here the central double line is again symmetrical but the lines on either side are asymmetrical and in the opposite sense on the two sides of the central one. Moreover, the asymmetry is considerably enhanced by the assumption that the velocity in the shell at any point is proportional to the radius vector from the nucleus to the point.

Spectral lines have been computed according to the foregoing picture, and compared with observation. Since the angle between the major axis and the line of sight is not known for any nebula, the comparison must be qualitative, but within this limitation the agreement between observed and calculated lines is excellent. Thus it is found that NGC 7009 is oriented so that the line of sight is nearly perpendicular to its true major axis. NGC 7662 on the other hand is viewed at a considerable angle to its major axis, and the assumption of an ellipsoidal velocity distribution gives the best agreement between calculated and observed lines.

The observations of the Orion nebula with the multislit and the measurements of the plates have been completed by Münch and Wilson. This study provides data on the motions of the gases over the brighter parts of the nebula. A large number of additional observations were made

of the shapes of the emission lines in the Orion nebula by Code and Wilson using the photoelectric spectrum scanner on the 100-inch telescope, special attention being given to N_1 , N_2 , and $H\beta$. The wings of these lines were traced out to the 0.1 per cent level in order to determine the velocity dispersion implied by the line wings. A continuous emission feature at approximately 5034 Å with a half-width of about 10 Å was found in the Orion nebula which cannot be identified with any blend of atomic lines. In this region faint lines were identified at $\lambda 5041$ and $\lambda 5057$ with the $4^2P^\circ - 4^2D$ multiplet of Si II and at $\lambda 5048$ with the He I line from the $2^1P^\circ - 4^1S$ transition. These lines are exceedingly weak, representing an intensity of only a few per cent of He I 5015.7, which in turn is only a few per cent of the N_1 line of [O III]. These relative intensities indicate one of the promising advantages of the scanning technique. In addition, well resolved measurements of the components of 3727 [O II] were obtained in faint extensions of the nebulosity, and the intensity ratios were in good agreement with Osterbrock's photographic determinations. $H\beta$ was traced out beyond the obvious nebulosity down to emission measures of the order of 300.

Radial Velocities

Minkowski has now finished his part of a program of radial velocities of planetary nebulae that was undertaken as a joint program by him and by Dr. N. U. Mayall at the Lick Observatory. Radial velocities of 142 new planetaries have been obtained from observations with the 100-inch and the 200-inch telescopes. A total of nearly 250 new radial velocities for faint and small planetaries has now been determined at the Mount Wilson and Palomar Observatories, or at the Lick Observatory, or at both. A plot of them shows that very large velocity dispersion occurs around the Galactic center, where the velocities range from -250 to $+300$ km/sec. The large dis-

person hides a regular radial-velocity variation of 8 km/sec per degree of longitude from negative to positive values, as the longitude increases and passes that of the Galactic center, for the planetaries clustered around the nuclear region. Generally negative velocities appear near the longitude of the apex, and positive ones around the antapex. This pronounced reflection of the sun's Galactic rotation, together with the inconspicuous radial-velocity variation in the nuclear region, may be an indication that the system of planetary nebulae has relatively small Galactic rotation, but the results of a complete analysis, which will be undertaken by M. Schmidt at Leiden, have to be awaited before such a conclusion can be definitely accepted.

Densities of Nebulae

Minkowski and Osterbrock made point-by-point density determinations in NGC 650/1 and NGC 6720, using the [O II] $\lambda 3727$ intensity-ratio method, to study the spatial structure of these two planetary nebulae. The outer shell of NGC 6720, originally discovered by Duncan, has a lower density and also an even more pronounced filamentary structure than the classical bright ring and center. These inner parts, in which the mean electron density is of the order of $10^3/\text{cm}^3$, are, however, also extremely filamentary. NGC 650/1 appears from these observations to be a nebula rather similar in structure to NGC 6720, but seen in a different projection on the plane of the sky.

Observations by Osterbrock of the $\lambda 3727$ intensity ratio in the Cygnus Loop showed that the electron density in the visible filaments of this nebula is of the order of a few hundred per cubic centimeter. The total mass of the whole visible filamentary nebula is probably of the order of 0.1 solar mass. The mass of un-ionized and therefore dark matter inside the loop must be considerably larger than the mass of the bright filamentary system.

Observations of the $\lambda 3727$ ratios in the small T Tauri nebulosity and in the brightest Herbig-Haro object showed that their electron densities are of the order of $10^4/\text{cm}^3$. These nebulae, both probably connected with stars in early stages of their evolution, differ from typical planetaries in having both [O I] and [O II] forbidden lines in their spectra. They therefore contain neutral oxygen, and hence neutral hydrogen, and the well known theory of radiative ionization, worked out by Strömgren, shows that they cannot be ionized in the ordinary way by ultraviolet radiation. It is possible, though not certain, that the mechanism of ionization is high-energy corpuscular radiation released by the involved stars.

Osterbrock and Miss Flather analyzed the electron-density distribution in the Orion nebula. A model was constructed, in which the density varies smoothly with distance from the center, and which reproduces in the mean the observations not only of $\lambda 3727$ ratios but also of the [O III]/[O II] and $\text{H}\beta$ /[O II] ratios. However, this preliminary model predicts much higher thermal radio emission by the Orion nebula than the published observations indicate. The apparent discrepancy shows that there must be very pronounced density fluctuations in the nebula, and though there is not enough observational information to specify these fluctuations completely, a model can be constructed that satisfies all the observations.

Expanding Shells around Novae and Supernovae

Since 1935 Baade has kept under observation at the 100-inch and later at the 200-inch the expanding shells around Nova T Aurigae (1890), Nova Persei (1901), Nova Aquilae (1918), Nova Cygni (1920), and Nova Herculis (1934). In order to conclude the series these novae have been reobserved under the best conditions during the past year. Together with simul-

taneous measures of the Doppler components of the expansion, these observations should furnish reliable data for the absolute magnitudes of five novae and provide a valuable check for the absolute magnitudes of novae based on the distance modulus of the Andromeda galaxy.

GLOBALAR AND GALACTIC CLUSTERS

For several years an extensive program has been in progress for the study of the color-magnitude relations and other properties of the stars in a number of globular and Galactic clusters. Such observations provide the basic data, about the properties of groups of stars of different ages, that are necessary to trace the evolution of stars having various initial masses.

Two extremely distant clusters, found by Abell on the National Geographic Society-Palomar Observatory Sky Survey, have been studied by E. M. Burbidge and Sandage. The clusters are numbers 3 and 4 of Abell's list of 13 new clusters. A series of plates of both clusters was obtained with the 200-inch in four observing seasons beginning in 1953. These plates, together with photometric transfers from Selected Areas 51 and 57, gave color-magnitude diagrams that clearly identify the clusters as globular. But the diagrams were abnormal along the horizontal branches. In neither cluster does the horizontal branch extend to the blueward side of the RR Lyrae domain; the stars are bunched at the red side of the horizontal branch. In this respect the clusters fit into the sequence of globular clusters ordered in the following way: M 13, M 10, M 2, M 92, M 15, M 5, M 3, Abell number 3, Abell number 4. Clusters near the M 13 end of the sequence have most of their horizontal-branch stars blueward of the RR Lyrae domain. Clusters near the M 3 end have horizontal branches equally populated blueward and redward of the RR Lyrae domain. If $M_v=0.0$ for the horizontal-branch stars, then the clusters Abell numbers 3 and 4 are 125,000 parsecs distant.

Baade also reobserved at the 200-inch the remnants of the supernova Ophiuchi of 1604. Intercomparison with a plate taken at the 200-inch in 1950 clearly showed motions in the nebulosity, but a much longer time interval will be necessary before a study of the motions becomes profitable.

This is twice the distance of the Magellanic Clouds and makes both clusters intergalactic.

Arp has pointed out that the sequence M 13, M 10, M 2, M 92, M 15, M 5, and M 3 also puts the globular clusters in order of decreasing mean period of their RR Lyrae stars. For example, the mean period of the Bailey type a and b variables in clusters like M 2, M 92, and M 15 is 0.64 day, but in clusters like M 5 and M 3 the mean period is only 0.53 day. Sandage has developed a partial theory to explain this and other differences in the RR Lyrae variables from cluster to cluster. It is believed that the absolute magnitudes of the horizontal branches of clusters are not the same. Therefore the absolute luminosities of RR Lyrae stars differ from cluster to cluster. Sandage's theory predicts that the difference in absolute magnitude is related to the difference in the mean period by $\Delta M_v = 3.0 \Delta \log P$. Therefore ΔM_v between the RR Lyrae stars in M 3 and M 15 must be 0.25 magnitude to explain the mean-period difference. Differences in the period-light amplitude and period-color relations predicted from the theory can be checked by observation of light-curves in the two colors B and V for variables in M 3 and M 15. Such a study for M 3 was completed and reported three years ago. The comparison study of variables in M 15 was started this year by Dr. Mary Connelley, of Mount Holyoke College, and Sandage. A long series of blue and yellow plates of M 15 were obtained with the 100-inch telescope during the report year. A two-color photoelectric sequence extending from $V=11.5$ to $V=17.2$ was also

obtained in M 15 with the 100-inch. The plates are being measured, and a check on the prediction of the theory will be possible.

Work on NGC 5897 by Sandage and Schmidt, on NGC 6712 by Sandage and Norton, and on NGC 6356 by Sandage and Wallerstein, reported last year, is nearly complete. Additional stars have been measured photoelectrically in each of these clusters with the 100-inch telescope. The three-color UBV photographic photometry of NGC 5897 by Schmidt shows that this cluster has a normal color-magnitude diagram of the M 13, M 92 type. There is a strong ultraviolet excess, which is normal for all globular clusters so far studied. Over 30 new RR Lyrae stars have been found in NGC 6712 by Norton from a series of 200-inch plates taken of this cluster in 1956. Norton is obtaining two-color light-curves of the variables and a color-magnitude diagram for the cluster. By far the most interesting cluster on the current program is NGC 6356. It is in a group that differs from halo clusters because the strength of the metal lines in the integrated spectrum is high. The group, first isolated by Morgan, contains NGC 6304, 6356, 6440, 6441, 6624, 6637, 6638, 6652, 6712, and 6838. With the exception of NGC 6838, all the clusters are very close to the direction of the Galactic nucleus. NGC 6356 has the very late spectral class of G5 and is the most readily accessible from these latitudes. A color-magnitude diagram by Sandage and Wallerstein for this cluster is nearly complete. They find no horizontal branch whatsoever to 4 magnitudes below the top of the giant branch. In this respect the color-magnitude diagram is similar to that of NGC 6838, which is another member of the group. The color-magnitude diagram for NGC 6838 has been obtained by Dr. W. Becker of Basel, Switzerland, and by Dr. J. Cuffey of Indiana University. Special spectroscopic and UBV photometric observations of the field around NGC 6356

are being made by Wallerstein to determine the interstellar reddening in front of NGC 6356 so as to check whether the intrinsic colors of the giant branch of this cluster are normal.

A preliminary survey of the magnitudes and colors of stars in M 13 was made by Baum in 1953. When the color-magnitude diagram was adjusted to place the cluster-type variables at 0.0 absolute magnitude it was found that the main sequence fell about 0.3 magnitude at the blue side of the standard main sequence. Because of the importance of this result for many theoretical problems a cooperative program was arranged between Dr. W. A. Hiltner of the Yerkes Observatory, Dr. Harold Johnson of the Lowell Observatory, and Baum and Sandage to extend the measurements to many additional stars of the cluster and thereby to reduce the statistical uncertainty. Many additional photoelectric measurements of B and V magnitudes have been made with the 200-inch by Baum and Johnson, and with the 42-inch of the Lowell Observatory and the 82-inch of the McDonald Observatory by Hiltner and Johnson. Sandage has measured and reduced B and V magnitudes of 250 faint stars in M 13 to locate the main sequence. Six plates in each color were measured and reduced by means of the extensive photoelectric sequence provided by the three photoelectric observers. The final results of the entire program are not yet definitive, but the main sequence does not appear to be as far from the standard main-sequence stars as the early results of 1953 indicated.

Thirty-nine stars in M 5 have been measured to fainter than $V=20$ by Arp photoelectrically in three colors in this near-by globular cluster. The photoelectric measures clearly define the complete color-magnitude diagram of M 5 from the brightest stars, down to the main sequence and including 2 magnitudes of that main sequence. As time permits, a few fainter stars will be measured; the observational

phase of this problem is nearly complete, however. Enough inner stars were measured so that all the previous measures on the bright part of the color-magnitude diagram can be converted to the BV system. A supplementary sample of fainter stars will be measured on photographic plates, and the ultraviolet colors will be analyzed.

The cluster NGC 2158 has been surveyed photoelectrically by Arp; despite its very sparse number of faint stars, it appears, from preliminary results, to have a globular-cluster-like color-magnitude diagram. The analysis of the globular cluster M 22 by Arp and Melbourne and the adjoining nuclear star field by Arp has been completed.

The results of a cooperative study of the Galactic cluster NGC 7789 by E. M. Burbidge, of Yerkes Observatory, and Sandage, reported last year, have been completed. NGC 7789 has a color-magnitude diagram like that of NGC 752 but much richer. The main-sequence break point occurs at $M_v \approx +2$, $(B-V)_0 \approx +0.35$. There is a Hertzsprung gap 0.4 magnitude wide extending from $(B-V)_0 = 0.4$ to $(B-V)_0 = 0.8$. From there, the giant sequence begins and extends to $M_v = -1.5$, $(B-V)_0 = 1.6$. The important point is that the giant branch intersects the giant sequence of M 11, which is a considerably younger cluster whose main-sequence break point occurs at $M_v = -1$. This result illustrates the funnel effect described earlier and is the strongest observational evidence suggesting that the masses of luminosity class III giant stars range from $1.2 \odot$ to $3.0 \odot$.

The color-magnitude diagram and luminosity function for the Galactic cluster NGC 188 have been completed by Dr. S. van den Bergh, of Perkins Observatory, and Sandage. A UBV photoelectric sequence of 54 stars extending from $V = 8.0$ to $V = 16.7$ was set up with the 60-inch telescope. A few additional critical observations of the five faintest stars were made by Arp with the 200-inch. Photographic

plates for the color-magnitude diagram were obtained with the 60-inch. Photographic plates for the luminosity function were obtained with the 48-inch Schmidt. The color-magnitude diagram has the same general shape as that of M 67. There is no Hertzsprung gap, and there is a 3-magnitude rise in the giant branch from the main-sequence termination point to the top of the giant sequence. But the important difference between NGC 188 and M 67 is the absolute magnitude of the main-sequence break point. The main sequence in NGC 188 terminates at least 0.5 magnitude fainter than M 67. On current evolutionary theories this means that NGC 188 is about 1.5 billion years older than M 67. But, to date, M 67 has been the oldest Galactic cluster known; its age has been taken as 5 to 6×10^9 years. If the present data for NGC 188 prove to be correct, we have a cluster whose age is 6.5 to 7.5×10^9 years. With the preliminary modulus which these first results indicate, the subgiant branch of the color-magnitude diagram for NGC 188 passes through the position of the field stars δ Eridani and μ Herculis in the H-R diagram. These stars have always been considered candidates for stars older than M 67 because in the diagram they are fainter than M 67. Additional observations on NGC 188 are being made to check the results.

Further photoelectric observations in the Galactic clusters NGC 2269, 2309, 2401, and 2453 were carried out by Schmidt. Owing to weather conditions this program is still unfinished. Photoelectric observations in the Galactic cluster NGC 6939 were completed and are being reduced.

In a joint program, Arp, Sandage, and Schmidt have measured photoelectrically about 100 stars in NGC 6940. This cluster is similar to the Hyades but richer. Work is continuing on fainter stars in this cluster, and Wallerstein is taking spectra in NGC 6940. Arp has measured about 40 stars photoelectrically in M 37, a Galactic cluster similar to the Hyades or NGC 752

but with a strong giant branch. Photographic plates of the cluster remain to be measured to complete the diagram.

Further photoelectric observations on the UBV system were made by Matthews of an unnamed cluster of early-type stars possibly connected with the Association I Camelopardalis. The cluster is situated in a region of very heavy obscuration which is responsible for a pronounced increase of 21-cm radiation.

White Dwarfs in M 67

Because of its great age M 67 should contain a considerable number of white dwarfs. Moreover, they should be within reach of the 100-inch and 200-inch telescopes, since the distance modulus of M 67 is only $m - M = 9.6$. Therefore in the winter of 1956-1957 Baade started at the 200-inch a search for white dwarfs in M 67 which was continued during the past season. Only the central area with a radius of 8 minutes of arc has been searched. Two sets of photographic, photovisual, and ultraviolet exposures were available. Similar sets of Selected Area 51 provided the necessary standardization.

Altogether 32 stars were found which have color indices $\leq +0^m.20$. They range in magnitude from about 19.5 photographic to the limit of the plates, which is close to $m_{pg} = 22.7$. There seems to be little doubt that these very faint blue stars are white dwarfs of M 67, since they stand out by their blueness among the stars of similar brightness. Actually the number of white dwarfs in the center area of M 67 may be considerably larger, since the color limit mentioned above is rather arbitrary. However, color alone becomes an increasingly dangerous criterion as one goes to larger color indices, where only the spectrum can decide whether one is dealing with a white dwarf or not.

Absolute Magnitudes of Cepheids

The discovery by Irwin (1955) followed by Kraft (1957) and van den Bergh (1957)

of cepheids in Galactic clusters has made it possible for the first time to derive fundamental parameters for at least some cepheids in our own Galaxy. By accurate three-color photometry of the cepheids and the clusters containing them it is possible to derive accurate absolute magnitudes and intrinsic colors of the cepheids. One of the primary purposes, of course, is to determine an absolute zero point for the period-luminosity relation, but normal yellow-blue and ultraviolet color indices will also be very important. It is even possible, using evolutionary theory, to derive the age of these cepheids, and also a better estimate of their masses. In order to obtain definitive results Arp and Sandage have undertaken to measure, with the 60-inch and 100-inch telescopes, color-magnitude diagrams of all clusters containing cepheids and the light-curves of the cepheids in them that can be reached from the North. Dr. R. P. Kraft of Indiana University has cooperated in an important part of this program by obtaining spectra in these clusters. Sandage has finished the results for the cepheid CF Cassiopeiae in the Galactic cluster NGC 7790. He is completing material for two more, CE Cassiopeiae A and B in the same cluster. Arp has completed the results for EV Scuti in NGC 6664. Arp, Sandage, and Miss Stephens have made measurements of DL Cassiopeiae in NGC 129. A few check measures are still needed. To complete the known group, the following cepheids, located in anonymous clusters, are being analyzed by Arp and Sandage: CV Monocerotis, XZ Canis Majoris, AO Canis Majoris.

Dynamics of Clusters

Oort made an extensive dynamical study of the globular cluster M 3 which he had started in Leiden in collaboration with Dr. van Herk. This work was based on star counts made by Sandage. Some plates were taken with the 48-inch schmidt telescope in order to extend the star-count

data to large distances from the center. The investigations have resulted in a fairly satisfactory theory of the cluster's struc-

ture. This analysis indicates that in the outer regions there must be a strong preference for radial motions.

GALAXIES

The Local Group

Miss Swope finished the work on the color-magnitude diagram of the Draco system using plates taken by Baade. In agreement with expectation the diagram is of the same type as that of the globular clusters of the Galactic halo. It also conforms to the general rule that large numbers of cluster-type variables (the Draco system contains about 300) can be expected only if the horizontal branch on the red side of the variable gap is strongly populated.

Four photoelectric measures have been made by Arp in Draco for the calibration of the above program. They indicate that the original photographically transferred magnitude scale is quite good, but that the color indices should be moved about 0.2 magnitude toward the red, making the Draco system fall more into line with globular-cluster color-magnitude diagrams.

During the past year the observations of the variable stars in the Ursa Minor system and the Leo II system were concluded by Baade. In the near future detailed data should be available about the variables and the color-magnitude diagrams of three dwarf E galaxies, to which may be added the Sculptor system now under investigation at the Radcliffe Observatory. It is also hoped that the series of observations of NGC 185 which Baade has carried out at the 200-inch since 1950 will be sufficient to determine the types of the variables in this much larger E galaxy. NGC 185 is too far away to reach the cluster-type variables, but it contains long-period red variables in large numbers. The investigation of these variables should provide much-needed data about the long-period variables of the Population II.

In a start on the reduction of the photographic plates of NGC 6822 taken over the years by many observers, Arp has ob-

tained thirteen photoelectric calibration stars bracketing the magnitude level of the cepheids.

A photoelectric sequence of about twelve stars going almost to $V=21$ magnitude has been established by Arp in the near-by dwarf galaxy in Sextans. Measurements on photographic plates of about fifty members enable a preliminary color-magnitude diagram to be plotted. The photoelectric sequence will be strengthened and carried to fainter magnitudes; more stars will be measured in the color-magnitude diagram, and photographic plates taken by Baade will be measured to obtain light-curves of the dozen or so cepheids contained in the system.

An investigation was continued by Code and Dr. T. E. Houck of Washburn Observatory on the luminosities of the brightest OB stars in near-by galaxies. Houck observed photoelectrically on a three-color system a number of bright blue stars in NGC 6822 and M 33, and spectra were obtained by Code of some of these objects with the prime-focus spectrograph on the 200-inch reflector. In general these objects resemble the most luminous early-type stars in our own Galaxy, although they all show strong broad H and K lines presumably of interstellar origin. The bolometric magnitudes of the brightest stars are in excess of -11^m . From spectra previously obtained by Code and Houck of objects in the Large Magellanic Cloud a search was made for analogous stars in our own Galaxy. A comparison of the Large Magellanic Cloud star HDE 269700 with the star ζ' Scorpii was made. The spectra of these two objects are very similar and indicative of an extremely high luminosity. From the presently accepted distance moduli of the Large Cloud and the I Scorpii association, respectively, a

visual absolute magnitude of -9^m is found for these two objects and a bolometric absolute magnitude of the order of -11^m . These stars show some evidence of incipient instability in the form of light-variation and P-Cygni emission. The stability to radiation pressure was discussed.

Although the spectra and colors of the early-type stars in the Large Magellanic Cloud suggest that these objects are substantially similar to those in our own Galaxy, the Small Magellanic Cloud data do not show these similarities. In general all the spectra obtained by Code and Houck in the Small Cloud possess weaker lines than corresponding Large Cloud objects, and the interpretation of these results as reflecting a lower metal abundance for the Small Magellanic Cloud is difficult to avoid. This important result appears to be in contradiction to the results of Thackeray and Feast.

Rotation and Dispersion of Stellar Velocities

Spectroscopic observations with the Palomar prime-focus spectrograph of the H II regions in M 81 were continued by G. Münch in order to improve the rotational-velocity-curve previously reported. In a spectrum of the nucleus of M 81 obtained in the yellow region strong nebular lines of [O III] were discovered. Another plate in the ultraviolet (dispersion 66 Å/mm) showed also the [O II] doublet and the [Ne III] lines at $\lambda 3868$ in emission. These lines are narrow and resemble those observed in H II regions, extending out to a distance of 4 seconds of arc from the nucleus. With Sandage's distance modulus $m-M=27.1$ the diameter of the emitting region would be 100 parsecs. The relative intensities of the [O II] doublet correspond to an electron density around 10^3 , decreasing slightly outward. The emission lines are inclined at an angle of $2^\circ 5' \pm 0^\circ 4'$ with respect to the direction of dispersion, indicating an angular velocity of 560 ± 40 km/sec per kpc. This value is quite close to

the angular velocity of the Galactic system at 0.11 kpc from the nucleus (445 km/sec per kpc, according to Kwee, Muller, and Westerhout). Tentatively it is believed that this emission nebula in the center of M 81 is of the same nature as the radio source observed in the center of the Galactic system.

Oort has cooperated with Minkowski in the investigation of velocity dispersions and rotations of elliptical and S0 galaxies, and of the amorphous parts of spirals, including in particular an extension of the dynamic study of the S0 galaxy NGC 3115 made by Oort in 1939. Such observations are essential for understanding the dynamics of elliptical galaxies and the initial conditions under which they were formed. They are also fundamental for finding the mass density in the universe.

Poor weather restricted the observations. Spectra of NGC 3115 indicate that the rotational velocity does not increase linearly with the distance from the nucleus as older results had implied. The new results suggest that up to about 50 seconds from the center the mass density may be approximately proportional to that of the light-emission, but that at greater distances the distribution of mass becomes practically homogeneous while the light-density continues to diminish rapidly. The inference rests on only three spectra and needs to be confirmed.

Magnitudes and Energy Curves of Galaxies

Elliptical galaxies provide the indicators by which the distances of clusters of galaxies are fixed. In order that proper correction may be made for evolutionary changes it is important that information about their stellar content be available.

During the past three years new six- and eight-color observations of galaxies of all types as well as a representative sampling of various kinds of stars have been obtained by Baum. The best synthesis thus far achieved has led Baum to the con-

clusion that 22 per cent of the visual light and 5 per cent of the total light (bolometric) of the large elliptical galaxies in Virgo come from Population II stars and the remainder from old Population I stars. The contribution of Population II in M 32 is somewhat larger. Three dwarf elliptical galaxies have now been measured photoelectrically with enough precision to show that they are not similar to the large ellipticals but have color indices like those of the globular clusters.

From data now in hand, the integrated color indices of the old stellar systems ranging from globular clusters at one extreme to large elliptical galaxies at the other have been plotted against absolute magnitude, and the results indicate a clear dichotomy. All old systems (that is, those that no longer produce new stars) brighter than -16 absolute magnitude are in the class dominated by old Population I, whereas those fainter than -14 absolute magnitude are in the class dominated by Population II. The transition from one class to the other occurs between -14 and -16 absolute magnitude. Roughly speaking, M 32 represents the smallest size in the old Population I class.

Tift has completed the study with multicolor photoelectric photometry of 57 galaxies; 8 galaxies were also observed in the infrared. The color system has been placed on an absolute energy basis. Dependence of color on nebular type is well marked. The color of the nuclei depends on the inclination of the galaxy in a different way in spirals and in ellipticals. The variation of color with radius also differs between FG-type and F-type galaxies. Synthetic models of five different types of nuclei have been computed. K nuclei can be represented by a mixture of old Population I and II stars; F, FG, and probably AF nuclei can be produced by adding various numbers of young blue stars to K galaxies. A-type galaxies appear to be completely dominated by young stars. Some polarization measurements were made in dark lanes of M 31 and M 81.

Observations of the energy distribution of galaxies were continued by Code with the photoelectric scanning spectrograph. In addition to the photoelectric scans, widened spectra of the nuclei have been obtained. The combined data of line profiles and continuum are being used to form synthetic stellar populations. A dip has been found in the continuum of ellipticals in the region of the Mg I $\lambda 5167-84$ triplet that is characteristic of giant-type spectra in contradistinction to dwarfs.

Distance Scale

A reanalysis of all steps required to establish the extragalactic distance scale has led Sandage to the conclusion that the Hubble expansion parameter, which relates redshifts and distances, is probably as low as $H=75$ km/sec per 10^6 parsecs. This value represents a total change of a factor of 7 in Hubble's 1936 scale of distances. The correction is composed of two parts. A correction of about a factor of 3 for galaxies within the local group comes primarily from the change in zero point of the cepheid period-luminosity relation (Baade, Blaauw and H. R. Morgan, and Mineur). The second factor of about 2 for distances beyond the range of cepheid variables (i.e., beyond the local group and the near-by M 81, M 101 groups) comes from the misidentification in 1936 of the H II regions for brightest stars. By filter photography, it has become clear that all the bright "resolved" knots in galaxies with velocities in the range from 600 to 2000 km/sec are H II regions like the Orion nebula rather than stars. The erroneous identification in 1936 gave distances that were too small. It is only because of the great technical advances of H α plates since 1936 that this situation has now been clarified.

As part of this examination of the distance scale, Sandage concluded that the period-luminosity relation of the cepheid variables is not as simple as previously believed. The physical relation for these

oscillating stars appears to be an equation between period, luminosity, and intrinsic color (or temperature), and the approximation of a three-parameter relation $f(P, M_v, B-V)$ by the two parameters of period and luminosity introduces intrinsic scatter. The hypothetical treatment predicts that the period-luminosity relation for cepheids should have an intrinsic spread of 1.2 magnitudes if read at a given period. Arp's two-color light-curves for a number of cepheid variables in the Small Magellanic Cloud confirm the theory in its general form, but more data are needed before the full implications of the results for the distance scale are known.

In a program to provide photoelectric scales in near-by galaxies that have extensive photographic coverage of the cepheid variables, bright local standards have been established by Arp and Sandage in NGC 2403, M 81, and M 101.

Velocities of Galaxies

In last year's Annual Report a procedure was briefly described by Baum by which both the redshifts and the magnitudes of remote galaxies can be determined by photoelectric photometry. Each galaxy is observed in eight colors, and the data are reduced to a plot of radiated energy as a function of wavelengths. If a galaxy is redshifted, this whole energy-distribution-curve is shifted toward longer wavelengths. In this manner, redshifts can be determined for galaxies considerably beyond the range that can be reached with the spectrograph. The photoelectric observations also provide a direct measure of bolometric magnitude.

A preliminary analysis has been made of the results thus far in hand. These include observations in six clusters of galaxies ranging from the near-by cluster in Virgo to one of the remotest known clusters with a redshift of 0.4λ . When the logarithms of the redshifts are plotted against bolometric magnitudes, the points (one for

each cluster of galaxies) all fall remarkably close to a straight line of slope 5.

Supernovae

The search for supernovae has been continued by Zwicky in cooperation with the Steward Observatory, the Lick Observatory, and the observatory of the University of Berne in Switzerland. Humason joined the group, after his retirement, searching in particular for supernovae in near and medium-distant clusters of galaxies (as far as the Corona Borealis cluster). Mr. H. S. Gates, continuing his search with the 18-inch schmidt telescope, found three supernovae in NGC 4374 (=Messier 84) in the Virgo cluster, NGC 1365 in the Fornax cluster, and NGC 5236 (=Messier 83). The search at Palomar was in part supported by funds made available by the National Science Foundation.

The light-curves, in different colors, of recent supernovae were followed as far as possible by Zwicky with the 200-inch prime-focus spectrograph; miscellaneous observers contributed plates obtained with the 48-inch schmidt telescope and the reflectors on Mount Wilson. Arp and Sandage have started a program to set up photoelectric scales near all galaxies in which supernovae have occurred. Thus far photoelectric sequences have been set up in NGC 3992 and NGC 4214. It is intended to continue the supernovae search vigorously for several years and to look also for supernovae in a few of the most distant clusters of galaxies for the purpose of a more reliable distance determination than is available at the present time.

Density of Gas Clouds

Minkowski and Osterbrock attempted to determine the electron density of the interstellar matter that emits the [O II] $\lambda 3727$ doublet in the elliptical galaxy NGC 1052. Though the two components are completely blended by high-velocity dispersion of the system, the mean wavelength of the blend was measured and

compared with the mean wavelength corresponding to various relative intensities of the components, and therefore to various electron densities. The result is that the intensity ratio is near the asymptotic low-density limit, and the density is therefore of the order of or less than $200/\text{cm}^3$ —a rather low value; from the strength of the line it can therefore be deduced that there is a large amount of ionized interstellar gas in this galaxy. NGC 1052 is an elliptical galaxy with a very strong $\lambda 3727$ line, and it is probable that in other ellipticals with weaker emission lines the density of ionized interstellar matter is even lower. Osterbrock continued observing other elliptical galaxies with emission lines, in particular NGC 4278, in which the lines are very strong. The plates are now being reduced.

Catalogue of Galaxies and Clusters of Galaxies

Herzog and Zwicky have continued their work on the positions and apparent photographic magnitudes of some 35,000 of the brightest galaxies as well as on the positions, populations, diameters, and estimated distances of about 10,000 clusters of galaxies. This work was partly supported by a grant from the Office of Naval Research.

Multiple Galaxies

The direct photography of multiple galaxies has been continued by Zwicky for the purpose of investigating the association of types and the relative luminosities. This project has included a study of the spectral types of members of multiple galaxies as well as their dispersion in radial velocities. A group of about 100 elliptical galaxies occupying an area near R. A. 1h 23m, Decl. $-1^\circ 34'$, and including objects NGC 535, NGC 538, NGC 541, NGC 543, NGC 545, and others, has been under special study for the determination of spectral types, of velocity dispersion, and of the possible rotation of the group

of galaxies as a whole. Humason has cooperated in the reduction of the data.

Spectra of Intergalactic Luminous Matter

Two successful attempts were made by Zwicky in January and in April to obtain the spectra of the very faint (23rd magnitude per square second, or less) extended luminous bridges and clouds of intergalactic matter that connect many widely separated galaxies.

The spectrum of a pair of galaxies with a faint bridge between them and a very long plume-like extension was obtained first. It proved to be a pure absorption spectrum with pronounced H and K lines as well as the G band, all in absorption, connecting the two galaxies and extending along the plume. It is expected that most of the luminous intergalactic bridges will prove to be of this type, that is, that they are composed of stars, presumably old stars. The apparent velocities of recession are about 6280 km/sec for the fainter galaxy and 6380 km/sec for the brighter one.

A special effort was then made to choose a luminous bridge that promised to show emission lines. The group at R. A. 11h 8m 5s, Decl. $+29^\circ 2' 24''$, seemed particularly promising for the purpose. In connection with this object V. A. Ambartsumian, on red and blue prints of the National Geographic Society-Palomar Observatory Sky Survey, had discovered that the southernmost tiny knot of the multiple galaxy (at the end of the stringlike luminous bridge) is extraordinarily blue. Excellent blue spectra were obtained with the slit covering the central irregular barred spiral, the elliptical or S0 galaxy south of it, and the thin bridge with the blue knot. Whereas the spiral proved to be quite blue, the elliptical galaxy gives a much redder continuous spectrum, but both galaxies show strong $\lambda 3727$ emission lines. The little knot, in spite of being much fainter than the two main galaxies, has a $\lambda 3727$ emission line almost as strong as those of

the main galaxies, which easily accounts for its extreme blueness. As a matter of fact, the $\lambda 3727$ line appears in emission all along the exceedingly thin and faint bridge from the bright elliptical galaxy to the small blue knot south of it. The apparent velocity of recession of the whole group is about 8700 km/sec.

In spite of the elevated brightness of

the night-sky glow, due to the present high solar activity, the spectra so far obtained of the luminous intergalactic matter are surprisingly distinct. It can therefore be anticipated that at low sky brightness, a few years hence, it will be possible to obtain significant information on the spectral types of many luminous intergalactic bridges.

RADIO SOURCES

Minkowski has continued the investigation of objects that might be associated with radio sources. Poor observing conditions have hampered the progress of this work. It is becoming increasingly certain that only a small fraction of sources, even of those with reliable positions, can be identified with optically observable objects. The conclusion is now quite definite that there are not many galaxies whose radio emission is intermediate between the weak emission shown by normal galaxies and the very much—perhaps 1500 times—stronger emission of a few. At present it is not possible to decide whether this means that most radio sources are exceedingly strong emitters, such as the colliding galaxies Cygnus A, or whether the generally accepted assumption that the majority of the radio sources are distant galaxies is wrong.

A large amount of time was spent by Matthews in completing the observations for a 21-cm survey from Galactic longitudes 190° to 270° . The reduction of the records is almost complete.

The well known controversy about the distance of the radio source Cassiopeia A—from the optical data a distance of about 500 parsecs had been inferred whereas the radio observations demanded a distance ≥ 3000 parsecs—was finally settled when Baade showed that the optical data lead in a straightforward way to the picture of an expanding shell of finite thickness. The outer surface of the shell has a velocity of 7440 km/sec, the inner a velocity of 6200 km/sec. Combining these figures with

the observed transverse motions of the shell one obtains for the distance of the shell $D = 3.42 \pm 0.27$ kiloparsecs, in agreement with the radio determination. It is also certain now that because of its high velocity of expansion the Cassiopeia A nebula must be regarded as the remnant of a type II supernova, the first supernova of this kind identified in our own Galaxy. From the transverse motions of the condensations in the shell the date of the outburst can be computed. It took place A.D. 1697 ± 14 years. Why the event was not noticed is thoroughly explained by the distance modulus of the supernova ($m - M = 12.6$) and the large absorption in front of it, which amounts to at least 5 magnitudes. It is therefore highly improbable that the supernova reached the third magnitude at its maximum. An interesting feature of the shell is the deceleration of the hemisphere turned toward us, which reaches amounts up to 16 per cent of the original velocities. In the direction toward us the shell is therefore moving into some interstellar gas. The gas must contain patches of very high densities, for intermingled with the moderately decelerated condensations are some which are practically completely decelerated and which are optically characterized by extreme reddening. They are the red bits of the nebula that can be photographed only in red light. They must have been decelerated extremely fast, since they have reached distances from the center of the shell that differ in no way from those of the undecelerated condensations. More-

over, in the present velocity diagram of the shell there is not a single condensation in such a state of fast deceleration. The Cassiopeia A nebulosity represents the first clear-cut example of the collision of a high-

velocity gas shell with the interstellar medium. It is probably a safe guess that the radio emission is a result of this collision involving very high velocities and masses comparable to that of the sun, if not larger.

THEORETICAL STUDIES

A theoretical investigation was conducted by Code of the properties of the very luminous OB stars observed by Code and Houck. The masses of these stars are of the order of $100 \odot$, and they are characterized by electron scattering as the main source of stellar opacity and by the dominance of radiation pressure. A limit theorem was derived for the amount of mass in the convective core. It is found that the bulk of the mass is in convection and well mixed. As the star consumes hydrogen in the convective core the core shrinks, the luminosity increases, and, since the chemical composition of the outer radioactive envelope does not change, the force due to radiation increases, expanding the envelope. It is suggested that later-type supergiants are produced by this mechanism, and an investigation of the stellar atmosphere of such structures shows it to agree well with the observed properties of luminous supergiants. These stars are found to be unstable to radial pulsations with a secular change due to the expansion. The pulsation may be correlated with the velocity variations observed by Abt.

An investigation on the rate of star formation as a function of time was

started by Schmidt. A comparison between the distribution of gas perpendicular to the Galactic plane and that of young stars suggests that the rate of star formation varies with the square of the gas density. It is then possible to compute the rate of star formation as a function of time by taking into account the depletion of the gas due to star formation and the ejection of gas into interstellar space during the transition from the giant to the white-dwarf stage. The present rate of star formation is found to be one-fifth of the average rate. The initial luminosity function, which is determined from the rate of star formation and the observed main-sequence luminosity function in the field, fits the observed distribution of luminosities in open clusters within the uncertainty. The helium abundance of the sun and of the interstellar gas at present can be explained if the average efficiency with which hydrogen is converted into helium and ejected at the end of the giant stage is 40 per cent. The theory gives at least a partial explanation for the nearly constant gas density observed in the plane over a large range of distance from the Galactic center.

INSTRUMENTATION

The coude spectrograph of the 100-inch telescope has been undergoing extensive revision during the past few years. A device similar to a record changer was first installed to make the interchange of gratings very rapid and safe; it permits the selection of the grating with the optimum dispersion and blaze angle for each object under study. At the same time the slit was replaced with a new, longer model.

During the current year two new cameras of 8-inch and 16-inch focal lengths were installed. Both are of the schmidt type with twice-through corrector plate and field flattener. The corrector plate of the 16-inch is of fused quartz to permit observations in the ultraviolet to the limit of atmospheric transmission. With the gratings normally used these new cameras provide dispersions of 40 \AA/mm and 20

Å/mm in the blue, in addition to the dispersions of 10 Å/mm, 4.5 Å/mm, and 2.9 Å/mm already available with the older cameras.

A preliminary model of a photoelectric spectrum-scanning device was tried out by Code and Wilson on the coudé spectrograph of the 100-inch telescope this year. It scans by rotating the grating, the photo-multiplier being placed behind a slit located in the focal plane of the 9-foot camera. With this linear dispersion of 2.9 Å/mm a resolution of 0.05 Å was practical. An image slicer replacing the entrance slit proved to be highly desirable, substantially reducing the fluctuations due to seeing; it also increased the efficiency considerably. Approximately 20 per cent of the flux in the seeing tremor disk was transmitted, representing an increase of approximately a factor of 10 over the use of a simple slit at this resolution for average seeing. Seeing compensation was employed using a monitoring photocell intercepting a fraction of the light passing through the first slit. The spectral region was isolated with glass and interference filters, and the electronic circuitry was similar to that described by Hiltner and Code. On the basis of a test program extending over 20 nights, changes were suggested, and a modified instrument is now being constructed. In particular it was found that accuracy of scanning could not be achieved by rotating the grating in its present bearings, and the scanning will be done by a small optical offset mechanism in front of the second slit. The spectral isolation for the monitoring cell will be obtained by picking off light in the focal plane of the spectrograph. Finally, the electronic circuitry is being modified to utilize the band-pass characteristics of a single amplifier.

In September 1957 a procedure was set up by Arp that enables all photoelectric

observations to be automatically reduced by an electronic computer. A program consisting of about 1000 instruction commands is read into the Datatron Digital Computer (Model 204) at the California Institute. The computer, starting with given values of atmospheric extinction, then transforms all observed deflections into magnitudes and colors outside the earth's atmosphere. A least-squares solution is made, using all standard stars present, to obtain the best transformation between the telescope-photometer system and the standard-star system. The extinction is then computed on the requirement that the residuals between observed and given standard magnitudes be a minimum. A least-squares solution for the extinction is performed, and all magnitudes and colors are recomputed using the new extinction value.

A second program, prepared in April 1958, introduced a weighting function for observations at low altitudes and is intended to give a more sensitive computation of the extinction. Program II is presently being used. It is able to handle 99 stars as a group, and requires about 10 minutes of actual computation time to make the solution for that many stars. The final magnitudes and colors are automatically typed out on permanent record sheets in three columns, giving: (a) standard magnitudes, if any; (b) first computed values; (c) second computed values. The sheets are headed by the given extinction and first computed transformation coefficients, and ended by the computed extinction and second set of transformation coefficients. In general the electronic computer permits more efficient tabular handling of data, higher accuracy, and a data processing about 10 times faster than that previously obtainable. About 1500 stars have been processed so far in this program.

GUEST INVESTIGATORS

The following programs have been carried out by guest investigators during the current year.

Dr. George O. Abell of the Department of Astronomy, University of California at Los Angeles, has continued his investigation of rich clusters of galaxies. He had determined the bright end of the luminosity function of galaxies in rich clusters by means of extrafocal photographic photometry with the 48-inch schmidt telescope. Complete sets of yellow and blue plates are now available for 33 of the 36 rich clusters selected for the study. The photometric reductions are in progress.

In a recent statistical investigation of the distribution of rich clusters, Abell found highly significant departures from a random cluster distribution. The results strongly suggest the existence of second-order galaxian clusters with a mean diameter (for the Hubble constant, $H=75$ km/sec per 10^6 parsecs) of 58×10^6 parsecs. Eight individual groupings that appear to be second-order clusters have a mean diameter of 50×10^6 parsecs, and a mean population of 14 rich clusters. It is estimated that 10^{16} solar masses is an upper limit to the mass of a typical second-order cluster. The corresponding maximum value to be expected for the root-mean-square velocity dispersion is of the order of 10^3 km/sec.

Dr. Dinsmore Alter of the Griffith Observatory continued his program for the study of various features of the moon's surface. Eighty direct photographs were obtained at the Cassegrain focus of the 60-inch reflector. About half of them were on II-O plates and the others on 1-N plates using a Pyrex filter with a 10 per cent cutoff near 7400 Å.

Dr. Jan Borgman of the Kapteyn Astronomical Laboratory at Groningen, Netherlands, carried out an investigation for the purpose of detecting faint, possibly intergalactic, variable stars in a field near the Galactic pole. A total of 60 plates was taken with the 48-inch schmidt during the

months of February, March, and April. Using the 60-inch, UBV photometry was carried out for a number of stars in SA 4 and 5, classified by Dr. Elvius, in order to investigate the possibility of using the ultraviolet excess as a "population index" as suggested by Dr. Nancy Roman.

An investigation of the diameters of meteor trails was carried out by Dr. A. F. Cook of the Harvard College Observatory. Eighteen trailed exposures, centered on the radiant of the Geminid meteors, were obtained with the 48-inch schmidt camera on the nights of December 11 and 12. The telescope was focused alternately on the stars and on the meteors. In three hours of exposure 33 meteors were photographed, of which one was sporadic and another possibly so. The seeing on the second night was essentially perfect and contributed nothing to the widths of the trails. Three meteors, of which two were in focus, were bright enough to allow densitometry of their trails. By visual inspection both in-focus trails show a width greater than the star trails.

Dr. T. E. Houck of the Washburn Observatory carried out, in collaboration with Code, an investigation of the properties of OB stars in near-by galaxies. Dr. Houck made three-color photoelectric measurements of these stars while Code investigated their spectra.

A search for flare stars was made by Dr. Hugh M. Johnson of the University of Iowa. On 24 plates taken in rapid succession with the 48-inch schmidt camera of the region of SA 57 near the north Galactic pole, of an Ophiuchus-Scorpius dark-cloud field, and of an intermediate field around SA 89, were found 141 sample flare- and "flicker"-stars. The term "flicker" is introduced because of the rather low-grade activity of the samples, which were at first blinked and then iris photometered. About 1 high-latitude field star in 2000, brighter than photographic

magnitude 20, detectably flickers at least once in 88 minutes.

A study of Galactic symmetric nebulae was also made by Dr. Johnson using existing 48-inch material.

Between July 1, 1957, and June 30, 1958, Dr. William Livingston of the University of California obtained a total of 144 magnetograms of the solar disk with the equipment at the 150-foot tower. One magnetogram was made each day that the weather and instrumentation permitted. The sensitivity was adjusted at 4 to 15 gauss per interval of the raster in order to obtain optimum sensitivity without causing confusion in the sunspot zone. Copies of the magnetograms have been sent to cooperating organizations as part of the International Geophysical Year program.

In addition to the above, 161 magnetograms were obtained of the polar regions with a higher sensitivity of about 1 gauss per interval of the trace. Systematic errors have been reduced as far as possible in an attempt to measure the magnitude and extent of these fields. The fall and winter observations have indicated that the south polar field has a reversed direction to that found in the preceding sunspot minimum. The north polar field has been more or less intermediate, suggesting the need for more observations before definite conclusions can be drawn. These findings agree with the observations made by H. D. Babcock at the Hale Solar Laboratory in Pasadena.

From September through December 1957 photoelectric observations were made with the 20-inch reflector on Palomar Mountain by Dr. R. Lynds of the University of California at Berkeley. These observations consisted of intercomparisons of the early B giants in η and χ Persei with the aim of discovering variability. Of twenty-two stars observed, seven were found to be definitely variable. Those for which the type is fairly certain are Oosterhoff 612, a probable β Canis Majoris star, and Oosterhoff 1586, an eclipsing variable.

In addition, five of the remaining fifteen stars showed evidence of variability.

During April and May 1958 eight early B giants were observed for variability with the Palomar 20-inch. These stars are from a list of 45 B stars north of $\delta = -30^\circ$, brighter than $V=7.0$, and having MK classifications similar to those of the known β Canis Majoris stars. Six of the stars observed show variability; it is too early to ascertain the type.

Dr. John Mathis of the Michigan State University studied the hydrogen/helium abundance ratio in the diffuse nebula NGC 604 in the spiral Messier 33. In August he obtained spectrograms of both NGC 604 and the Orion nebula using the 60-inch telescope. The spectrograms indicated that the helium/hydrogen ratio in NGC 604 is 0.13 by number of atoms, almost exactly that found in the Orion nebula. NGC 604 showed an anomalous intensity ratio of $H\beta$ and $H\gamma$, however, indicating that the nebula might be self-absorbed in the hydrogen lines. If NGC 604 is self-absorbed, the helium abundance is less than that quoted above.

The cooperative program with the McMath-Hulbert Observatory has continued throughout the year. Observations with the Snow telescope were carried out by Mr. Thomas K. Jones.

The programs with the Snow telescope were closely integrated with the programs of the McGregor Tower Telescope of the McMath-Hulbert Observatory. Because of an incredibly poor observing season at Mount Wilson during 1957-1958, the Snow observations have lagged seriously behind the Lake Angelus observations. The Snow telescope was used on only 85 days.

The observing programs remain, because of the poor season, much the same as last year: (1) Systematic observation of He, 10830 Å, and K, 3934 Å, lines, at the limbs of the sun and in plage regions. (2) Systematic observation of $H\alpha$ on the driest days to eliminate the effects of water vapor. (3) Abundance observations in support

of the McGregor Tower program. (4) Absorption-coefficient observations in support of the McGregor Tower program. (5) Continuation of wavelength measurements in the $5\ \mu$ region. The results from the Snow telescope have been used entirely for the support of the detailed programs of the McGregor Tower.

During the current year Dr. D. H. McNamara of Brigham Young University continued his investigation of RZ Scuti. He finds the hydrogen lines split into two components, the stronger of which originates in a shell of gas surrounding the system, whereas the weaker component is the normal absorption line. He finds, also, that the individual helium lines give different rotational velocities, the weakest lines giving the largest. Both the hydrogen and helium lines are consistent with a hypothesis of a shell surrounding the star whose rotational velocity decreases with the height above the star.

Dr. McNamara has also obtained several plates of U Cephei that show incipient emission filling in the $H\alpha$ absorption line during phases near 0.25 and 0.75 P. With the 60-inch telescope he has also obtained spectrograms of faint B stars (magnitude 10-12) in the η and χ cluster in Perseus for the purpose of measuring the equivalent widths of the $H\alpha$ and $H\delta$ absorption lines. Plates have also been obtained of a number of sharp-line B_1 and B_2 stars in order to test for small radial-velocity variations. For θ Ophiuchi the radial-velocity observations indicate a small period variation in velocity of 3h 24m.

A revised system of classification of forms of galaxies has been devised by Dr. W. W. Morgan of Yerkes Observatory. This system, based on the relative importance of the nuclear concentration of light, has a fairly close correlation with the integrated spectral types, and permits interpretation in terms of the stellar population of the galaxies. It complements that of Hubble.

A total of 608 galaxies was classified on the revised system from direct photographs

in the Hubble-Sandage collection. A discussion of this material suggests that the stellar populations differ systematically in various nearer regions of the universe. For example, the percentage of "young-star rich" galaxies is high in the Ursa Major Cloud; it is considerably lower in the Virgo Cloud. In the nuclear region of the latter, there is a concentration of eight galaxies brighter than the 12th magnitude (Shapley-Ames), all members of which are in the "young-star deficient" category.

It now appears feasible to investigate stellar populations for all clusters of galaxies whose members are bright enough to permit widened spectra to be obtained having dispersions of the order of 300 Å/mm.

Mr. Luis Münch of the Tonantzintla Observatory obtained about 100 additional spectrograms of the OB stars in the associations I, II, and III Cassiopeiae. The plates are being measured for radial velocities, which, with the previously determined spectroscopic parallaxes, will be used for a study of the spatial relationship of these associations.

Dr. G. J. Odgers of the Dominion Astrophysical Observatory made an extensive series of observations of four β Cephei stars. Forty-six spectrograms of β Cephei, 48 of 12 Lacertae, 106 of HD 199140, and 30 of HD 202253 were obtained in rapid succession for studies of the short-period changes in the velocity displacement and intensities of the lines. The star β Cephei showed clear evidence of variable $H\alpha$ emission throughout the radial-velocity cycle. Strong $H\alpha$ emission was observed both in HD 199140 and in 12 Lacertae. Large radial-velocity discontinuities appear in the velocity-curves of both these stars of an amount equal to the semi-amplitude. Evidence of velocity differences with respect to H and He on the one hand, and Si, C, and N on the other, especially near the phase of radial-velocity discontinuity, was obtained in HD 199140.

Dr. Daniel Popper of the University of California at Los Angeles continued his observations and analyses of the orbits of

eclipsing binaries. Attention was given to problems of bolometric corrections and the stellar temperature scale, both of which are needed for a complete analysis of eclipsing systems. The usual system of bolometric corrections and of effective temperatures was improved considerably by the use of photoelectric colors such as $B-V$ instead of spectral types as argument. Numerous observations of photoelectric colors and magnitudes of stars were made with the 20-inch reflector on Palomar Mountain in connection with these problems.

Infrared spectra of Venus taken by John Strong and William M. Sinton at Palomar in 1953 exhibited a strong absorption band at $11.1\ \mu$. This band has been tentatively attributed to polymerized carbon suboxide. The existence of the gas, which has the formula C_3O_2 , and its polymerization products, has also been suggested by G. P. Kuiper to explain the cloud-layer color and polarization data of Venus. To ascertain the presence of carbon suboxide through its absorption bands between 3000 and 3300 Å, Dr. Sinton of the Lowell Observatory secured three plates containing seven spectra of Venus on April 5-6, 1958, with the 60-inch Cassegrain spectrograph. Visual inspection of these plates does not reveal the presence of C_3O_2 . Its presence or absence will be made more definite when the plates have been densitometered and compared with plates made of the sky spectrum on the same date.

An investigation of stars of spectral type F2 and earlier in a north Galactic pole region was made by Dr. Arne Slettebak of the Perkins Observatory in an effort to discuss the relative space densities of Population I disk stars and Population II halo stars as a function of the distance from the Galactic plane. This program originated from an earlier one carried out at the Hamburg Observatory in which a finding list of 601 such stars to about the 14th magnitude was obtained by means of objective-prism spectra.

On 19 nights at the 60-inch with the X-spectrograph and the 4-inch camera and on 3 nights at the 100-inch with the Newtonian spectrograph and the 3-inch camera, slit spectrograms were obtained for 125 Galactic pole and standard stars. A preliminary inspection of the spectrograms showed a number of Population II horizontal-branch stars and subdwarfs as well as a number of apparently normal Population I stars at very great distances from the Galactic plane. Accurate spectral classifications and radial velocities for all these stars will eventually be obtained.

Drs. Otto Struve and Jorge Sahade of the Berkeley Astronomical Department of the University of California carried out extensive observations of close early-type spectroscopic binaries. The main purpose of the investigation was to trace the evolution of the binaries, and to discover to what extent the interaction between the component stars and their loss of mass influences their evolutionary trends. Beta Lyrae was observed in the red and the near infrared to ascertain whether lines of a late-type component appear in these regions; the results were negative; the large atlas of the spectra of β Lyrae has been completed during the year. The emission at $H\alpha$, arising from a common envelope, was discovered in the system AO Cassiopeiae. Rapid changes in the structure of the He I line at $\lambda 6678$ were discovered on the spectrograms of the primary component of α Virginis. The observations of the eclipsing variable 29 UW Canis Majoris have disclosed emission at $H\alpha$ and remarkable changes in intensity and profile in the emission lines of N III and He II. Several observations were made of the eclipsing variable Y Cygni. Beta Persei (Algol) was observed at principal eclipse in the red and near-infrared regions in an attempt to detect lines of the secondary component of the eclipsing pair, but with negative results.

Measurements of the radial velocities of ϵ Aurigae on all Mount Wilson coude

spectrograms obtained since the 1928-1929 eclipse were completed by Dr. Struve. An extensive spectrophotometric study of some of the best recent spectrograms has been made by Dr. Margherita Hack.

An investigation of the luminosity function and radial density distribution in the Galactic clusters M 67, NGC 7789, NGC 188, and Praesepe was undertaken by Dr. Sidney van den Bergh of the Perkins Observatory. During eight nights in January and February, 170 plates were obtained with the 48-inch schmidt. A series of plates of each cluster were taken, twelve in the blue and one in the red plus two transfer exposures of Selected Areas. The blue exposures ranged from 4 seconds to 10 minutes.

A magnitude sequence was determined in each cluster with the aid of an Eichner photometer. It was then used to find the limiting magnitude of each plate. The luminosity function and the radial density distribution were next determined by counting the number of stars in concentric rings about the nucleus.

The luminosity function of M 67 was found to be deficient in faint stars, and the faint stars are located predominantly in the outer regions of the cluster. NGC 7789 is probably the richest Galactic cluster known. The faint stars are again located predominantly in the outer part of the cluster. The main-sequence population of the cluster is deficient in faint stars in comparison with the Salpeter luminosity function, although not to the same extent as M 67. The Praesepe was found to contain some hundreds of stars with absolute visual magnitudes between +7.0 and

+11.5. The cluster radius for the faint stars is larger than for the bright ones.

From August 21 to August 31, inclusive, Dr. Peter van de Kamp of the Sproul Observatory of Swarthmore College made a visual and photographic search with the 100-inch reflector for close faint companions of a number of near-by stars. The observing conditions were adverse. A number of persistent elongations were noted visually, using magnifying power up to $1000\times$. None were considered sufficiently reliable to be regarded as positive evidence for companion stars. For future exploration twenty plates were taken with exposures ranging from 1 second to 15 minutes.

Dr. Merle Walker of the Lick Observatory made photoelectric observations of AE Aquarii in cooperation with Code on two nights during August 1957. Dr. Walker observed the star at the 60-inch, obtaining a continuous record of the light-variations in the ultraviolet. Simultaneously observations in the Balmer continuum, in H emission lines, and in the continuous spectrum between lines were obtained by Code with the photoelectric scanner at the 100-inch. The reduction of the 60-inch observations is complete, and correlations are being made with the continuous UV traces to determine how the various features of the spectrum change during the rapid light-variations of the star. This program is a continuation of the program of simultaneous photoelectric and spectroscopic observation of the star reported earlier by Deutsch and Dr. Walker.

STAFF AND ORGANIZATION

Dr. Walter Baade retired from the staff of the Observatories on June 30, 1958. On coming to the Mount Wilson Observatory in October 1931, Baade attacked a series of photometric problems. They included the establishment of photometric standards in Selected Areas, investigations of

the light-curves of supernovae, and studies of the magnitudes of variables in globular clusters and in galaxies and the use of these magnitudes for distance determinations.

During World War II Baade took advantage of the darker skies due to the

black-out of the Los Angeles area and the development of faster red-sensitive plates to make a critical investigation in the red of M 32, NGC 205, NGC 147, NGC 185, and the nucleus of the Andromeda galaxy. His plates achieved resolution of these objects for the first time and showed that the brightest stars in them are the red giants. This finding led Baade to introduce the concept of population types. Population I, whose most prominent features are the blue giant stars, is found in the neighborhood of our sun and in the spiral arms of the Andromeda and other spiral galaxies; Population II, which is characterized by the red giants, is observed in the elliptical galaxies and the nuclei of the spirals and in globular clusters. Detailed studies were then made of the color-magnitude relations in representative samples of the two populations by Baade and his collaborators. The results of these investigations provide the observational basis for present theories of stellar evolution which suggest that the difference between the population types is primarily one of age.

With the completion of the 200-inch Hale telescope Baade continued his studies of the stellar content of the Andromeda galaxy and the other members of the local group. Special attention was given to cepheid variables and other stellar types that might be used as distance indicators for fixing the distances of these objects. These studies represent the first step in the precise determination of the distances of all objects outside the Milky Way. Baade's observations provided much of the evidence for the revision of the absolute magnitudes of the cepheid variables and the resultant increase in the distance scale of all objects outside our own Galaxy by a factor of nearly 3.

Baade also collaborated with Minkowski in the identification of many of the radio sources with optically observed objects and in the physical interpretation of the nature of these sources.

Dr. Baade served on the Observatory

Committee from 1953 until the time of his retirement.

Dr. J. Beverly Oke was appointed to the staff at the end of the report year.

Research Division

Staff Members

Halton C. Arp
Walter Baade¹
Horace W. Babcock, *Assistant Director*
William A. Baum
Ira S. Bowen, *Director*
Arthur D. Code²
Armin J. Deutsch
Jesse L. Greenstein
Fred Hoyle
Rudolph L. Minkowski
Guido Münch
J. Beverly Oke
Donald E. Osterbrock³
Robert S. Richardson³
Allan R. Sandage
Olin C. Wilson
Fritz Zwicky

Research Associates

Paul W. Merrill
Jan H. Oort
Richard v. d. R. Woolley

Carnegie Research Fellows

Robert F. Howard
Thomas A. Matthews
Maarten Schmidt

Research Fellows

David R. Dewhirst
H. Lawrence Helfer
Leona Marshall
Neal Tanner
George Wallerstein
Volker Weidemann

Research Assistants

Sylvia Burd
Mary F. Coffeen
Thomas A. Cragg
Dorothy S. Deutsch
Edith Flather
Emil R. Herzog
Joseph O. Hickox

¹ Retired June 30, 1958.

² Resigned August 31, 1958.

³ Resigned June 30, 1958.

A. Louise Lowen
Mildred Matthews
Cynthia Stephens
Henrietta H. Swope
Carol Tift³

Student Observers

Walter K. Bonsack
Daniel E. Harris
William G. Melbourne
Robert G. Norton
William G. Tift
Robert L. Wildey

Editor and Librarian

Alexander Pogo

Photographer

William C. Miller

Instrument Design and Construction

Lawrence E. Blakeé, Electronic Technician
Floyd E. Day, Optician
Kenneth E. DeHuff, Machinist
Robert D. Georgen, Machinist
Don O. Hendrix, Superintendent, Optical Shop
Melvin W. Johnson, Optician
Bruce Rule, Project Engineer
Oscar Swanson, Instrument Maker
Russell R. Van Devender, Jr., Designer and Superintendent, Instrument Shop

Maintenance and Operation

Mount Wilson Observatory and Offices

Audrey A. Acrea, Stewardess
Paul F. Barnhart, Truck Driver

Ashel N. Beebe, Superintendent of Construction
Wilma J. Berkebile, Secretary
Ernest V. Cherry,¹ Janitor
Hugh T. Couch, Carpenter
Stewart F. Frederick, Janitor
Eugene L. Hancock, Night Assistant
Mark D. Henderson, Gardener
Anne McConnell, Administrative Assistant
Leah M. Mutschler, Stenographer and Telephone Operator
Bula H. Nation, Stewardess
Alfred H. Olmstead, Night Assistant
Arnold T. Ratzlaff, Night Assistant
Clyde Sanger,¹ Gardener
John E. Shirey, Janitor and Relief Engineer
Benjamin B. Traxler, Superintendent

Palomar Observatory and Robinson Laboratory

Fred Anderson, Machinist
Dorothea Davis, Secretary
Eleanor G. Ellison, Secretary and Librarian
Ferd Feryan, Mechanic⁴
Arlis R. Grant, Stewardess
Leslie S. Grant, Relief Night Assistant and Mechanic
Byron Hill, Superintendent
Charles E. Kearns, Night Assistant
Harley C. Marshall, Office Manager
George W. Pettit, Janitor
Robert E. Sears, Night Assistant
William C. Van Hook, Electrician and Assistant Superintendent
Gus Weber, Assistant Mechanic

⁴ Died June 11, 1958.

BIBLIOGRAPHY

- Abell, George O. The distribution of rich clusters of galaxies. *Astrophys. J., Suppl. Ser.*, 3, 211-288 (1958).
- Arp, Halton C. Cepheid variables in the Small Magellanic Cloud. *Astron. J.*, 63, 45 (1958).
- Baade, Walter. Large-scale structure of spiral nebulae. In *Intern. Astron. Union Symposium 5*, ed. by N. G. Roman, pp. 1-3, Cambridge, University Press, 1958.
- Babcock, Harold D. Arthur Scott King, 1876-1957. *Pubs. Astron. Soc. Pacific*, 69, 333-335 (1957).
- Babcock, Harold D., and Wm. C. Livingston. Changes in the sun's polar magnetic field. (Abstract.) *Science*, 127, 1058 (1958).
- Babcock, Horace W. The sun's general magnetic field. *Nature*, 178, 533 (1956).
- Babcock, Horace W. Identification of solar "M" regions with unipolar magnetic regions. *Astrophys. J.*, 126, 224 (1957).
- Babcock, Horace W. A catalog of magnetic stars. *Astrophys. J., Suppl. Ser.*, 3, 141-210 (1958).
- Babcock, Horace W. Stars as sources of cosmic rays. *Phys. Rev.*, 109, 2210-2211 (1958).
- Baum, William A. Review of *Scientific Uses of Earth Satellites*, ed. by J. A. Van Allen, *Pubs. Astron. Soc. Pacific*, 69, 368-369 (1957).
- Baum, William A. Rapport de la Sous-commission des convertisseurs d'images. In *Draft Reports*, Moscow meeting, August 1958, *Intern. Astron. Union*, pp. 60-69, Cambridge, University Press, 1958.
- Baum, William A., W. K. Ford, Jr., and J. S. Hall. Recent astronomical tests of thin-film

- image converters. (Abstract.) *Astron. J.*, 63, 47-48 (1958).
- Bonsack, Walter K. Radial-velocity variations in four short-period variable stars. *Astrophys. J.*, 126, 291-301 (1957).
- Bonsack, Walter K. Wavelength variation in the spectrum of 56 Arietis. *Pubs. Astron. Soc. Pacific*, 70, 90-97 (1958).
- Bowen, Ira S. Instrumentation at the Mount Wilson and Palomar Observatories. *Pubs. Astron. Soc. Pacific*, 69, 377-384 (1957).
- Bowen, Ira S. The universe from Palomar. *Griffith Observer*, 22, 69-78 (1958).
- Bowen, Ira S. Astronomy in a changing world. In *Frontiers in Science*, ed. by E. Hutchings, Jr., pp. 289-294, New York, Basic Books, 1958.
- Burbidge, E. Margaret, and G. R. Burbidge. Chemical composition of the Ba II star HD 46407 and its bearing on element synthesis in stars. *Astrophys. J.*, 126, 357-385 (1957).
- Burbidge, E. Margaret, and G. R. Burbidge. Cosmical alchemy. In *Frontiers in Science*, ed. by E. Hutchings, Jr., pp. 180-187, New York, Basic Books, 1958.
- Burbidge, E. Margaret, G. R. Burbidge, W. A. Fowler, and F. Hoyle. Synthesis of the elements in stars. *Revs. Modern Phys.*, 29, 547-650 (1957).
- Burbidge, E. Margaret, and A. Sandage. Properties of two intergalactic globular clusters. *Astrophys. J.*, 127, 527-538 (1958).
- Burbidge, Geoffrey R. Acceleration of cosmic-ray particles among extragalactic nebulae. *Phys. Rev.*, 107, 269-271 (1957).
- Burbidge, Geoffrey R. Particle energies and magnetic energy in the Crab Nebula. *Astrophys. J.*, 127, 48-53 (1958).
- Burbidge, Geoffrey R., and F. Hoyle. Anti-matter. *Sci. American*, 198 (no. 4), 34-39 (1958).
- Burbidge, Geoffrey R. See also Burbidge, E. Margaret.
- Code, A. D., and T. E. Houck, A superluminous B-type star in the Large Magellanic Cloud, and its galactic counterpart. *Pubs. Astron. Soc. Pacific*, 70, 261-266 (1958).
- Cragg, Thomas. Solar activity in 1957. *Pubs. Astron. Soc. Pacific*, 70, 299-302 (1958).
- Cragg, Thomas. See also Nicholson, Seth B.
- Deutsch, Armin J. A method for mapping the surfaces of some magnetic stars. (Abstract.) *Astron. J.*, 62, 139 (1957).
- Deutsch, Armin J. Recent observations of the companion of Mira. (Abstract.) *Astron. J.*, 63, 49 (1958).
- Ford, W. K., Jr. See Baum, William A.
- Fowler, William A. See Burbidge, E. Margaret.
- Greenstein, Jesse L. Non-thermal emission in the cometary nebula NGC 2261. In *The Non-stable Stars*, pp. 86-87, Erevan, Acad. Sci. Armenian S. S. R., 1957.
- Greenstein, Jesse L. Possible energy sources of T Tauri type stars. In *The Non-stable Stars*, pp. 104-112, Erevan, Acad. Sci. Armenian S. S. R., 1957.
- Greenstein, Jesse L. Theoretical problems of discrete radio sources. In *Radio Astronomy: Intern. Astron. Union Symposium 4*, ed. by H. C. van de Hulst, pp. 179-191, Cambridge, University Press, 1957.
- Greenstein, Jesse L. Studies of the white dwarfs. III. The recurrent nova WZ Sge as a probable white dwarf. *Astrophys. J.*, 126, 23-27 (1957).
- Greenstein, Jesse L. The spectra of the white dwarfs. In *Handbuch der Physik*, ed. by S. Flügge, 50, pp. 161-186, Berlin-Göttingen-Heidelberg, Springer-Verlag, 1958.
- Greenstein, Jesse L. Draft report of the President of the IAU Commission 29, Stellar Spectra. In *Draft Reports*, Moscow meeting, August 1958, Intern. Astron. Union, pp. 247-263, Cambridge, University Press, 1958.
- Greenstein, Jesse L., and M. S. Matthews. Studies of the white dwarfs. I. Broad features in white dwarf spectra. *Astrophys. J.*, 126, 14-18 (1957).
- Greenstein, Jesse L., and W. J. Luyten. Studies of the white dwarfs. II. Some new white dwarfs. *Astrophys. J.*, 126, 19-22 (1957).
- Greenstein, Jesse L., M. Hack, and O. Struve. The spectroscopic binary BD+74°493. *Astrophys. J.*, 126, 281-290 (1957).
- Greenstein, Jesse L., R. F. Sanford, and F. Zwicky. On the absolute magnitude of Nova Puppis 1942. *Pubs. Astron. Soc. Pacific*, 69, 352-353 (1957).
- Greenstein, Jesse L., and P. C. Keenan. Abundances of metals, CN, and CH in giant stars. *Astrophys. J.*, 127, 172-190 (1958).
- Greenstein, Jesse L., and G. Wallerstein. The helium-rich star, Sigma Orionis E. *Astrophys. J.*, 127, 237-240 (1958).
- Greenstein, Jesse L. See also Merrill, Paul W.; Swings, Pol; Tift, William G.
- Hack, Margherita. See Greenstein, Jesse L.
- Hall, J. S. See Baum, William A.
- Herzog, E., P. Wild, and F. Zwicky. A new catalogue of bright galaxies and clusters of galaxies. *Pubs. Astron. Soc. Pacific*, 69, 409-420 (1957).
- Houck, T. E. See Code, A. D.
- Hoyle, Fred. Forecasting the future. In *Frontiers in Science*, ed. by E. Hutchings, Jr., pp. 117-121, New York, Basic Books, 1958.
- Hoyle, Fred. The place of technology in civilization. In *Frontiers in Science*, ed. by E. Hutchings, Jr., pp. 268-277, New York, Basic Books, 1958.

- Hoyle, Fred. See also Burbidge, E. Margaret; Burbidge, Geoffrey R.
- Joy, Alfred H. Flare spectra in dwarf stars. In *Non-stable stars: Intern. Astron. Union Symposium 3*, ed. by G. H. Herbig, pp. 31-34, Cambridge, University Press, 1957.
- Joy, Alfred H. SS Cygni type variables as spectroscopic binaries. In *The Non-stable Stars*, pp. 137-140, Erevan, Acad. Sci. Armenian S. S. R., 1957.
- Joy, Alfred H. Henry Norris Russell. *Griffith Observer*, 21, 86-89 (1957).
- Joy, Alfred H. Walter Sydney Adams. *Biographical Memoirs, Natl. Acad. Sci.*, 31, 1-31 (1958).
- Keenan, Philip C. See Greenstein, Jesse L.
- Livingston, William C. See Babcock, Harold D.
- Luyten, Willem J. See Greenstein, Jesse L.
- Matthews, Mildred S. See Greenstein, Jesse L.
- Merrill, Paul W. Walter S. Adams, observer of sun and stars. *Science*, 124, 67 (1956).
- Merrill, Paul W. Walter Sydney Adams (1876-1956). *Year Book, Am. Phil. Soc.*, 103-105 (1956). See also *Monthly Notices Roy. Astron. Soc.*, 117, 243-244 (1957).
- Merrill, Paul W. Symbiosis in astronomy. *Mém. soc. roy. sci. Liège*, 20, 436-448 (1958); in *Étoiles à raies d'émission*, Les congrès et colloques de l'Université de Liège, 7, 436-448 (1958).
- Merrill, Paul W. From atoms to galaxies. *Astron. Soc. Pacific Leaflet* 349, 8 pp. (1958).
- Merrill, Paul W., and J. L. Greenstein. Double absorption lines in the spectrum of R Andromedae. *Pubs. Astron. Soc. Pacific*, 70, 98-101 (1958).
- Minkowski, Rudolph. Optical investigations of radio sources. Introductory lecture, 1955 Jodrell Bank symposium. In *Radio Astronomy: Intern. Astron. Union Symposium 4*, ed. by H. C. van de Hulst, pp. 107-122, Cambridge, University Press, 1957.
- Minkowski, Rudolph. The problem of the identification of extragalactic radio sources. *Pubs. Astron. Soc. Pacific*, 70, 143-151 (1958).
- Münch, Guido. Study of the hydrogen and helium II lines in a high-temperature subdwarf. *Astrophys. J.*, 127, 642-657 (1958).
- Münch, Guido. Review of *Non-stable Stars: Intern. Astron. Union Symposium 3*, ed. by G. H. Herbig. *Pubs. Astron. Soc. Pacific*, 70, 226-227 (1958).
- Newburn, Ray L., Jr. The RR Lyrae stars in NGC 4147. *Astron. J.*, 62, 197-203 (1957).
- Nicholson, Seth B. The solar cycle. *Astron. Soc. Pacific Leaflet* 348, 8 pp. (1958).
- Nicholson, Seth B., Th. Cragg, and others. Summary of Mount Wilson magnetic observations of sunspots for March, 1957, to February, 1958. *Pubs. Astron. Soc. Pacific*, 69, 358-362, 469-473, 578-583 (1957); 70, 114-119, 212-217, 319-324 (1958).
- Osterbrock, Donald E. Parallel filamentary structure in diffuse nebulae. (Abstract.) *Astron. J.*, 62, 144-145 (1957).
- Osterbrock, Donald E. Electron densities in filamentary nebulae. *Pubs. Astron. Soc. Pacific*, 70, 180-184 (1958).
- Osterbrock, Donald E. Review of *The Restless Universe*, by Max Born, transl. by W. M. Deans. *Pubs. Astron. Soc. Pacific*, 69, 372 (1957).
- Richardson, Robert S. Spectroscopic observations of Venus for rotation made at Mount Wilson in 1956. *Pubs. Astron. Soc. Pacific*, 70, 251-260 (1958).
- Sahade, Jorge, and G. Wallerstein. The spectrum of Algol in the near infrared at principal eclipse. *Pubs. Astron. Soc. Pacific*, 70, 207-208 (1958).
- Sandage, Allan. Observational approach to evolution. III. Semiempirical evolution tracks for M 67 and M 3. *Astrophys. J.*, 126, 326-340 (1957).
- Sandage, Allan. The birth and death of a star. *Am. J. Phys.*, 25, 525-531 (1957); in *Frontiers in Science*, ed. by E. Hutchings, Jr., pp. 188-189, New York, Basic Books, 1958.
- Sandage, Allan. Current problems in the extragalactic distance scale. *Astrophys. J.*, 127, 513-526 (1958).
- Sandage, Allan. See also Burbidge, E. Margaret.
- Sanford, Roscoe F. See Greenstein, Jesse L.
- Smith, Harlan J. See Tift, William G.
- Struve, Otto. See Greenstein, Jesse L.
- Swings, Pol, and J. L. Greenstein. Presence of forbidden lines of oxygen in cometary spectra. *Compt. rend. acad. sci., Paris*, 246, 511-513 (1958).
- Tift, William G., and J. L. Greenstein. MWC 603, a high-velocity symbiotic star. *Astrophys. J.*, 127, 160-171 (1958).
- Tift, William G., and H. J. Smith. T Sextantis, an RR Lyrae star of type "c." (Abstract.) *Astron. J.*, 63, 54 (1958).
- Walker, Merle F. The extremely rapid light-variations of old novae and related objects. In *Non-stable Stars: Intern. Astron. Union Symposium 3*, ed. by G. H. Herbig, pp. 46-56, Cambridge, University Press, 1957.
- Wallerstein, George. Note on the behavior of the RV Tauri-type star No. 11 in Messier 2. *Astron. J.*, 62, 168 (1957).
- Wallerstein, George. Movement observations on the Greenland Ice Sheet. *J. Glaciol.*, 3, 207 (1958).
- Wallerstein, George. Radial velocities and spectral characteristics of the Population II cepheids M 5 No. 42, M 5 No. 84, and TW

- Capricorni. *Astrophys. J.*, 127, 583-590 (1958).
- Wallerstein, George. *See also* Greenstein, Jesse L.; Sahade, Jorge.
- Wild, P. *See* Herzog, E.
- Wilson, Albert G. Astronomy and eschatology. In *Frontiers in Science*, ed. by E. Hutchings, Jr., pp. 205-213, New York, Basic Books, 1958.
- Wilson, Olin C. He emission in late-type stars. *Astrophys. J.*, 126, 46-50 (1957).
- Wilson, Olin C. Limitations on the physical theories of H and K emission lines. *Astrophys. J.*, 126, 525-528 (1957).
- Wilson, Olin C. Review of *Astrophysics I: Stellar Surfaces—Binaries*, vol. 50 of the *Handbuch der Physik*, ed. by S. Flügge. *Pubs. Astron. Soc. Pacific*, 70, 332-334 (1958).
- Zwicky, Fritz. Non-uniformities in the apparent distribution of clusters of galaxies. *Pubs. Astron. Soc. Pacific*, 69, 518-529 (1957).
- Zwicky, Fritz. Propellants for tomorrow's rockets. *Astronautics*, Aug. 1957, pp. 45-49, 95-96.
- Zwicky, Fritz. News on gravitation and nuclear goblins. *Griffith Observer*, 22, 49-63 (1958).
- Zwicky, Fritz. The first shots into interplanetary space. *Eng. and Sci.*, 21 (no. 4), 20-23 (1958).
- Zwicky, Fritz. The morphological method and vector associations. In *Les méthodes de la science moderne*, pp. 311-317 (French transl., pp. 319-326), Paris, Editions Science et Industrie, 1958.
- Zwicky, Fritz. *See also* Greenstein, Jesse L.; Herzog, E.

COMMITTEE ON IMAGE TUBES FOR TELESCOPES

Cooperative Project of Mount Wilson and Palomar Observatories, Department of Terrestrial Magnetism, National Bureau of Standards, and United States Naval Observatory

W. A. BAUM, *Mount Wilson and Palomar Observatories*

JOHN S. HALL,* *United States Naval Observatory*

L. L. MARTON, *National Bureau of Standards*

M. A. TUVE (*Chairman*)

Department of Terrestrial Magnetism

* After September 1, 1958, Director, Lowell Observatory, Flagstaff, Arizona.

The Committee on Image Tubes for Telescopes has continued during the past year to encourage the development of photoelectric image-intensifying devices for use in astronomy. Working toward this goal the Committee has fostered research in promising schemes for the production of image intensification and tried to evaluate new image-tube hardware produced for the Committee by various industrial research and development groups. Attention has been directed both to methods that record the photoelectrons themselves and to methods that use photoelectrons to produce an optical output.

During the report year, three barrier-film image tubes were tested with some success on the Naval Observatory's 40-inch telescope at Flagstaff, Arizona. Electrons ejected from the photocathode of a tube of this type are directly recorded when they impinge upon a very fine-grained emulsion; the cathode is protected from contamination by a barrier film approximately 1000 Å thick through which the photoelectrons (after being accelerated through 10 kv) can pass. Such a system derives its advantage over direct photography from the photocathode's higher quantum efficiency implemented by the high storage capacity of fine-grained nuclear-type emulsions. This principle is discussed in earlier reports (e.g., Year Book 54, 1954-1955, pp. 39 ff.), and the previous work on thin-film image tubes is described in last year's report (Year Book 56, p. 79).

The early tests of this type of tube were complicated by the task of removing the glass cap (which protects the thin barrier film from rupturing under atmospheric pressure) by cracking the glass with an electrically heated wire after the tube is mounted in a vacuum chamber. We have circumvented this difficulty by having the tube equipped with a metal cap and by using a mechanical "can opener" in the vacuum chamber in which the tube is mounted. This system has worked well

on the one dummy tube and the three live tubes tested this year.

In August 1957, a thin-film tube having a very poor cathode (a photosensitive area only 4 mm wide and 10 mm long and a sensitivity of less than 1 microampere per lumen) was used to record the images of some bright stars and some laboratory test patterns. Estimates were made of the thermionic background introduced by the photocathode and of the resolution capabilities of the tube. The tube was operated for three nights with little loss in sensitivity before the vacuum pumps were turned off and the photocathode allowed to decay.

In November a thin-film image tube having a photosensitivity of about 5 microamperes per lumen was tested. Provision was made for partial cooling of the cathode to reduce the spurious background due to thermionic emission and for placing the fine-grained emulsion about 40 microns from the barrier film to reduce the loss of resolution due to the scattering of the electrons by the thin barrier film. The components of double stars 5 seconds of arc apart could be resolved when this was done. A number of 30-second exposures were made on an open cluster, M 36, and compared with direct photographs of M 36 taken on 103a-D emulsions. Microphotometer tracings indicated that the signal-to-noise performance of the image tube was roughly on a par with that obtained by short-exposure photography. The same image density would have been obtained in a much shorter exposure if the cathode of the image tube had been of average sensitivity.

The useful exposure time of this tube for individual photographs was limited to less than 1 minute by the background emission from the S1 (infrared-sensitive) cathode. In order to extend the useful exposure time, the equipment was modified so that the cathode could be cooled with liquid nitrogen. This reduced the spurious back-

ground in a tube tested in February to the extent that, with exposure times of 1 minute, no background exposure could be detected.

These preliminary tests indicate that the barrier-film tubes are potentially useful devices for astronomical work, even though the method is somewhat encumbered by the continuously pumped vacuum system required on the telescope. The Committee is continuing their further development.

A more convenient system of image intensification would be one in which electrons originating from a photocathode impinge upon a phosphor screen and the resulting optical image is recorded by conventional photography. To do this with a net gain over direct photography, however, the photoelectrons must be multiplied inside the image tube, thereby increasing the brightness of the image on the phosphor screen to compensate for the inefficient process of photographing the screen.

Two methods of producing this internal intensification are being explored. One of them involves a series of thin membranes, each having a phosphor on one side and a photocathode on the other. At present two companies are trying to overcome the technical difficulties of making this type of tube, but no samples suitable for telescope tests were yet available at the end of the report year.

The second method involves a similar series of thin membranes that function as secondary-electron emitters. As in the first method, the electrons must be focused from the photocathode to the first membrane, from each membrane to the next, and from the last membrane to the phosphor screen. Development of this type of secondary-electron image intensifier is now being carried forward by another company.

Both these methods appear promising, but the technical difficulties that still exist in the fabrication of the tubes must be overcome before this type of image intensifier can be evaluated under the conditions encountered in astronomical work. The Committee also looks forward to future tests on similar tubes with internal cascade intensification, and with electrical output, which could have many advantages if the signal-to-noise ratio is satisfactory.

The National Science Foundation made a grant to the Committee to support more fully the industrial costs and the testing program now required for the development of image-intensifying tubes for use in astronomy. This grant will supplement earlier appropriations for the task of extending the range of telescopes to fainter objects, both in direct photography and in spectrographic measurement. The Committee acknowledges the vigorous and effective contributions made by W. Kent Ford, Jr.

DEPARTMENT OF TERRESTRIAL MAGNETISM

Washington, District of Columbia

MERLE A. TUVE, *Director*

CONTENTS

	<i>page</i>		<i>page</i>
<i>Introduction</i>	95	Atomically polarized ion source	
<i>Experimental Geophysics</i>	97	(APIS)	125
Radio astronomy	97	Biophysics	127
Precise position apparatus.....	97	Introduction	127
Radio emission from the sun.....	98	Fractionation methods	129
Flux measurements of radio sources.	99	Properties of ribosomes.....	132
Satellite observations at 40 and 108		Kinetics of incorporation into mac-	
mc	100	romolecular fractions	138
Moon reflections	100	Virus purification	142
Other activities	101	Incorporation of amino acid analogs	
Radio hydrogen	101	into bacterial proteins.....	143
The earth's crust.....	104	Cell-free systems	148
Seismic studies	106	The metabolic pool problem in <i>E.</i>	
Mineral age measurements.....	111	<i>coli</i>	149
<i>Theoretical and Statistical Geophysics</i> ...	117	Synthesis in mouse tissue.....	155
Equatorial electrojet	117	<i>Hydra</i> and <i>Planaria</i>	157
Cosmic-ray investigations	118	<i>Operations and Staff</i>	162
<i>Laboratory Physics</i>	122	Cooperative work of the Department.	162
Nuclear physics	122	Administration and operation.....	163
Coulomb excitation of xenon.....	122	<i>Bibliography</i>	163
Level structure of Na ²²	123	<i>Personnel</i>	165



The Carnegie Andes Expedition for the International Geophysical Year occupied more than 200 observing sites during 1957 in Peru, Bolivia, and Chile. By observations on the waves from large explosions in open-pit copper mines, a beginning was made in determining the nature of the deep rock structures of subnormal density which cause the elevation of these huge mountain masses. About half of the observing locations were above 14,000 feet, on the spectacular high plateau of the Andes.

INTRODUCTION

Basic research is not a business activity. It is a highly personal activity, a quest, rooted in devoted personal interest. It grows out of individual initiative, and its progress is guided and modified by the investigator himself. Thus, the work of a basic research laboratory is not designed by administrators as a comprehensive program, and it correspondingly fails to exhibit a clear and stepwise progression toward a recognized end result. There is no plan or expectation of an end result; there are to be recorded only incidents of fresh recognition or opportunity as the investigator hews out a new path in a recognized direction.

Research is not measured by the attainment of predicted goals but is observed as a process of individual development and fulfillment in a man's relationship to ideas and his tests of their validity. A later process, a bit to one side of the central creative effort, concerns the wider sharing of his own testing of ideas with others, for whatever common use they may serve. But most investigators do not find it satisfactory to work strictly alone. Scientific knowledge and current research activity are today so broad and yet so intensive that no one individual finds in himself all the information and all the stimulus needed to go ahead effectively, even on one narrow front of interest, and it is natural for a loose and free association into small, informal groups to occur among like-minded colleagues. This has happened also here in the Department.

Our areas for research activity have been selected by a process of discussion and on the basis of a mutual agreement among several individuals, or at least between the investigator and one other critical colleague, that a given area, related to physics in some intelligible way, offers opportunities for fresh query and new illumination concerning process and order in nature.

This report is then the record for one year of the research activities of a modest group of men whose basic allegiance and training are in physics, and whose enthusiastic interests range over a number of fields where a background in physics provokes questions that can be formulated in clear terms. The detailed research topics concern physical and chemical processes governing the behavior of matter and energy under such widely differing circumstances as obtain in the tenuous gas clouds of interstellar space or in the fantastically compact and orderly cosmos which is the nucleus of a living cell.

The International Geophysical Year has naturally been a great stimulus to our staff. The IGY, 1957-1958, was conceived some years ago in discussions among members and former members of this Department. Instead of undertaking a great expansion of staff and expenditures in order to operate expeditions to the Antarctic or elsewhere to help carry out the United States national program, and thus altering the highly personal character of our research activities here, our efforts have been restricted to specific enlargements of research activities already in hand, using our present staff and some (foreign) collaborators. Two of these enlarged projects were given additional support by generous grants from the National Science Foundation, on recommendation of the U. S. National Committee for the IGY.

An expedition to the high Andes of Peru, Bolivia, and Chile, comprising ten men with six specially equipped vehicles, undertook to measure the waves from large explosions in open-pit copper mines. There, in an effort to measure some of the major characteristics of the structure of the earth's crust in those spectacularly elevated regions, in several months of work the expedition made valuable observations

along the flank of the Andes, but found unusually drastic attenuation of the waves under the high Andes plateau. The reconnaissance was therefore only partly successful. Indications were obtained that the volcanic and metamorphosed crust of the earth is thicker near and under the Andes than under the Rocky Mountains, for example, but these explosion-wave results in South America must be augmented by observations on local earthquakes. Preparations for a collaborative program of earthquake studies are under way with university colleagues in Peru and Chile.

The program of isotope age measurements of igneous rocks, especially those of Precambrian age, has been given extra emphasis and geographical coverage as a part of our IGY contribution. Field trips for rock samples were made in South America and in western and northern Europe, and several special field trips through eastern, northern, and western United States, to collect samples from selected areas. It was also arranged to extend and enlarge our previous collaborative studies of locations in Australia and Africa, and for visiting investigators to work with us here.

The network of five magnetic stations set up in Peru to observe the equatorial "electrojet" in the high atmosphere for the IGY, as described in the report for last year, has been in operation during much of this report year. The data are of particular interest during periods of magnetic disturbance.

The nuclear physics work here has recently started off in a fresh direction. It has been clear for some time that great analytical advantages would accrue in experiments which utilized a "polarized" beam of protons, a beam in which the proton spins are lined up. During this year arrangements have been made with colleagues at Yale University for a joint effort to produce a beam of polarized protons

using our large Van de Graaff generator here. The 6-meter diameter of the high-voltage electrode gives ample space for installing a magnetically split beam of the Rabi type, to replace the customary ion source at the top of the high-voltage tube. The required apparatus is still under construction as the report year ends.

Observations of the hydrogen clouds among the stars of our galaxy were systematically carried forward during much of this year, using our 54-channel recorder and the Würzburg parabola for observing the radio emission at 1420 megacycles per second; preparations also went ahead for installing the 60-foot parabola being constructed for us by the Blaw-Knox Company. A comprehensive survey of the hydrogen clouds nearest the sun (large apparent diameter) was carried well toward completion, and detailed records for about half of the equatorial (Milky Way) areas were obtained. Solar activity was observed with special radio antenna arrays, and performance tests were begun of the extended arrays constructed and installed this year for precise radio positions, with a view to added optical identifications of celestial radio sources.

The biophysics group has been concerned with studies of the sites and rates of synthesis within the living cell of nucleic acids and of certain specialized proteins. The articulation of the synthetic mechanisms, and the distribution of these activities among the morphological components of the bacterial cell, continue to be the focus of interest here, following naturally in sequence after the previous comprehensively analytical studies of amino acid synthesis and incorporation. Observations of the detailed but finite specificity of synthetic mechanisms and the corresponding degree of variation in the resulting proteins, using chemical analogs of amino acids, have constituted another area of special interest and activity.

EXPERIMENTAL GEOPHYSICS

RADIO ASTRONOMY

*B. F. Burke, W. C. Erickson,¹ J. W. Firor,
H. L. Helfer,² H. E. Tatel,³ M. A. Tuve,
and H. W. Wells*

PRECISE POSITION APPARATUS

Testing and construction have proceeded on a 400 mc/sec linear array suitable for measuring positions of radio sources to within a few square minutes of arc. The previous report (1956-1957) described the preliminary tests that led to the present array, which consists of a pair of V reflectors, each 614 feet long, arranged on an east-west baseline with a spacing of 1842 feet between centers. The arrays can either be used separately, giving a fan beam $\frac{1}{2}^\circ \times 20^\circ$ to half-power points, or together as an interferometer with a lobe spacing of 4 minutes of arc. The calculated gain indicates that the two arrays should have the effective collecting area of two 90-foot paraboloids. Since it is contemplated to make measurements at several different azimuths, the antennas were built in sections to facilitate moving. Each section is a 60° V, 8 feet on a side and $9\frac{1}{2}$ feet long, fed by four collinear full-wave dipoles. All the elements are connected by a symmetrical branching feeder system with open-wire transmission line used throughout. At the close of the year, all the elements had been mounted and final connections were being made.

An essential test was performed on the phase stability of open-wire transmission lines, a vital factor in the accuracy of the final measurements. The extent to which atmospheric conditions, such as temperature, humidity, frost, and dew, would limit the use of open-wire lines was unknown when the present program was undertaken, although it was expected that the lines would be usable for a considerable

fraction of the time. During a 2-week period in November 1957, measurements were made that confirm these expectations and provide a quantitative estimate of the accuracy to be expected.

Absolute electrical line length was not important, since balanced feeder systems were planned for the arrays, but it was vital that the distances from the center of the line to either end remain equal, within the desired limits of tolerance. Most of our measurements were made on a line 1900 feet long, consisting of two parallel no. 6 copper wires, with a spacing of 3 inches, and supported every 50 feet by nylon filaments. Short circuits were placed at the ends, and at the center a 385 mc/sec signal was fed into each half. By comparing the relative phases of the two reflected waves, the difference in electrical lengths of the two halves could be measured, assuming that all the reflected wave came from the short circuit at the end. By replacing the short circuit with a matched termination, it was possible to measure the reflected wave from irregularities, droplets of water on the line, and similar spurious effects. Using this system, a change in line balance of 2 parts per million could be detected, although spurious reflections, determined by measurements with the matched load, limited the final accuracy to 1 part in 50,000. In general, the balance was either very good or very bad, a difference of more than 1/12,000 being considered bad. During the 228 hours of measurement, 64 per cent of the time the balance was better than 1/45,000, corresponding to an angular accuracy (when used in an array for measuring radio source positions) of about 4 seconds of arc. Seventy-five per cent of the time the balance was better than our desired tolerance of 1/12,000. The short-term stability was excellent, most of the periods of 1/45,000 stability being many hours long. Serious unbalance, that is, greater than

¹ Now with Convair Scientific Research Laboratory, San Diego, California.

² Now with Mount Wilson and Palomar Observatories, Pasadena, California.

³ Deceased November 15, 1957.

1/12,000, occurred only at times of heavy frost, heavy dew, or falling rain.

Consequently, open-wire lines can be expected to give many consecutive hours during which their phase stability should be sufficiently good to use in our position measurements of radio sources. Perhaps one-quarter of the time the phase unbalance will be too great, but the unbalance will be shown on the monitor, and at such times other components of the system, such as the antennas themselves, would probably also be suspect. (*B. F. B., J. W. F.*)

RADIO EMISSION FROM THE SUN

The solar fan beam array described in last year's report has been operating now for over a year, and enough records have been obtained to describe the types of events occurring on the sun at a wavelength of 88 cm (340 mc).

The most common feature of the sun at this wavelength is the quiet bright spot which was illustrated by some scans in our report last year. Such a spot produces a flux at our antenna ranging from the smallest that can be recognized, about 5×10^{-23} watt/meter²/cycle/sec, up to about 5×10^{-22} . The positions on the solar disk usually agree with that of large plages, but other, equally large, plages apparently have no radio bright spot associated with them. The radio spots that do occur persist for several days or sometimes for a complete disk passage.

There seems to be no clear distinction between the quiet bright spots and the other common feature of the 88-cm sun, the active spots. The active spots, although persisting for several days like the quiet ones, show changes in intensity and produce frequent small bursts, lasting a second or less. It is not uncommon for a quiet spot to become active during a disk passage. (A burst-producing region was also illustrated in the previous report.) The active spots can be much more intense than the quiet ones; the largest so far seen

gave a steady flux of 60×10^{-22} with instantaneous values during the bursts of perhaps twice this value.

Two comparisons have been made of the positions of active and quiet spots with those seen at other wavelengths. Some scans of the sun made at 21-cm wavelength have been published by the Australian group, and similarly, the results of scans made at 177-cm wavelength have been published by the French group. Neither of these sets of scans was made simultaneously with our 88-cm scans, but for spots persisting for several days, and if proper allowance is made for solar rotation, the comparisons should still be of value.

At the shorter wavelength, 21 cm, the features of the sun are almost entirely quiet bright spots agreeing in position with practically all large plages. There must be, then, 21-cm bright spots with no 88-cm counterpart, and the comparison of the records quickly verifies this conclusion. But the more surprising result is that the active spots at 88 cm frequently agree in position with 21-cm quiet bright spots. If borne out by more extensive comparisons, this fact provides the first link between the regions seen at 21 cm, which all evidence indicates are the result of relatively hot, dense regions in the lower corona, and the noise storm bursts of the long wavelengths, which are almost certainly nonthermal in origin.

The comparison with the 177-cm scans shows that, whereas many noisy regions are seen in common, some very intense features at the longer wavelength are absent at 88 cm. The conclusion is that there are disturbed regions high in the solar atmosphere which do not extend to the lower levels sampled by the 88-cm radiation.

At present, comparisons are in progress with groups in many countries, including measurements at wavelengths of 177, 88, 21, 9.8, 8, and 3 cm, and at optical wavelengths. (*J. W. F.*)

87-Megacycle Christianson Array

A survey was conducted at Derwood to determine the optimum recording frequency for a Christianson array at these longer wavelengths. Tests over periods of several weeks showed that the sun (and other radio sources) could be observed without serious interference using a frequency of 87.3 mc (345 cm).

Preparations are being made for the installation of this array at the Seneca,

tensity measurements on discrete radio sources. All observations have continued to apply the same principles of maximum simplicity to achieve minimum uncertainty in the results.

The status of "color index" of radio stars is somewhat improved since last year, partly as a result of the efforts of URSI Commission V at the Boulder meetings in August 1957. The high level of solar activity, however, has continued to keep

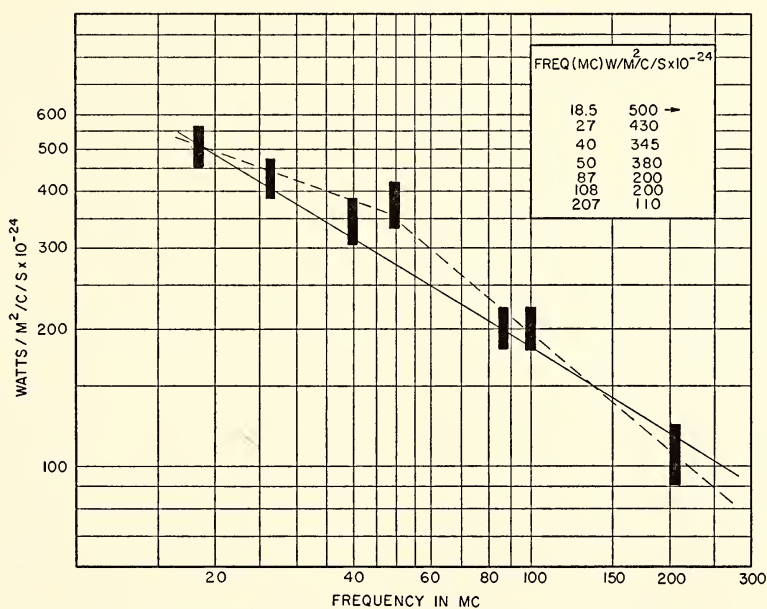


Fig. 1. Flux of Cassiopeia A, Derwood, Maryland, CIW-DTM, June 1958.

Maryland, site using a 3000-foot east-west baseline. Sixteen antenna elements and branching open-wire feeder systems are being fabricated. Tests have been made on several types of antennas, and a simple 90° corner reflector has been found to be most suitable. The instrument will have fruitful application to studies of "halo" or related effects of radio sources in addition to the solar observations. (H. W. W.)

FLUX MEASUREMENTS OF RADIO SOURCES

The report of last year outlined the basic objections and techniques of absolute in-

investigators from returning to the spectrum below 30 mc, which has been the region of greatest disagreement.

Further calibrations of the diode noise generators reveal excellent agreement with thermal sources up to frequencies somewhat greater than 100 mc, but at 200 mc the instruments were found to be in error by factors of 2 or 3.

Our experience with satellite recording at 40 mc demonstrated the feasibility of observing the strong radio sources using receivers with narrow band widths, say 1 to 5 kc. The result is an ability to make

flux measurements in parts of the spectrum (that is, 40 mc) that were completely masked by interference with the former broad-band (100 kc) receivers. Before obtaining a flux value for Cassiopeia A at 40 mc the series of measurements extending from 18.5 to 207 mc strongly suggested a distribution as shown by the dashed line of figure 1. Its characteristic feature is a "break" or change in slope at about 50 mc. The addition of our 40-mc value, however, would suggest a potential straight-line "color index" as shown by the solid line of the figure.

These developments clearly indicate a need to verify our earlier results at 50 mc. Intercomparisons with other investigators show our 50-mc results to be more "in agreement" than our 40-mc values. Any change in slope as suggested by the dashed line of figure 1 should not necessarily be interpreted as a basic change in the spectrum as generated. More probably it would result from modification in color index imposed by dust or other particles between the source and the earth, the absorption becoming more pronounced at the lower frequencies. Another possible effect of absorption in interstellar space may be a net over-all change in slope of the spectrum of a particular source with no observable "breaks." Either type of spectrum has interesting possibilities for development of a scale of distance (or perhaps a measure of galactic dust) but must await the precise establishment of color indices for several discrete radio sources. (H. W. W.)

SATELLITE OBSERVATIONS AT 40 AND 108 MEGACYCLES

Within a few hours of the launching of the first Soviet satellite on October 4, 1957, we were recording its signals at 40 mc. An interferometer designed for 38 mc was quickly converted to the satellite frequency, and the first transit was recorded at 22^h25^m EST, October 4 (03^h25^m GMT, October 5). The continued monitoring of

its signals through October 25 led to discovery of an "image" effect and to other interesting propagation phenomena.

In addition to the characteristic interference pattern of normal satellite transits there were a few distinct recordings at times when the satellite was on the *opposite side of the earth near an antipodal point*. This effect has been described as a radio "image" or "ghost" satellite.

Although "images" are well established in optical and electrical theory, it is difficult to offer a very satisfactory explanation of the effect. A review of the literature revealed that a basic theory for antipodal focusing was developed by Professor B. van der Pol of Holland in 1919. Any theoretical or physical explanation of the image effect appears to require a stable, homogeneous atmosphere with considerable dependence on the position or height above earth of the transmitter.

Subsequent recordings of the second and third Soviet satellites at 40 mc have failed to produce any additional "images." Contributing factors are believed to be the greatly reduced transmitter power and substantially different orbits.

At 108 mc the signals from United States satellites were also monitored for unusual propagation effects. Although over a period of several months we have not identified any radio images there is reason to anticipate substantial data on atmospheric refraction and absorption when the recordings are analyzed. (H. W. W.)

MOON REFLECTIONS

A short series of experiments were conducted in cooperation with Stanford University and the Evans Signal Laboratory. Transmitting stations at Stanford or Evans Signal Laboratory beamed signals on the moon at 106 to 108 mc. When received with a conventional phase-switching interferometer the moon signals appeared as an artificial radio source showing the characteristic lobe structure. In addition to the moon signals, tests with the Evans

Signal Laboratory (distance: 150 mi) showed a number of spurious interference patterns thought to be reflections from high-flying aircraft. Other applications of these techniques have been discussed.

Calculations based on known parameters of the experiments show the effective radar cross section of the moon to be 10^5 sq km, which is about 1 per cent of its actual cross section. (H. W. W.)

OTHER ACTIVITIES

A phase-switching interferometer at 38 mc has been installed and operated at the National Radio Astronomy Observatory, Green Bank, West Virginia. The instrument is intended to provide an independent check on certain radio star observations. It has also been useful for demonstration purposes and as a means of assessing the level of radio interference in the area.

A search has been made for Jupiter signals at 40 mc. Several months of records at times normally favorable to the reception and identification of Jupiter signals have been examined. The results are entirely *negative*, no signals having been identified at this frequency. (H. W. W.)

RADIO HYDROGEN

The density distribution and velocity distribution of atomic hydrogen gas clouds in our galaxy continue to be of major interest to our radio-astronomy group. Attempts to observe atomic hydrogen (by the radio line emission at 1440 mc/sec) in more distant objects, whether by absorption (as for Cygnus A) or by emission, has been postponed until we can have the use of a parabola larger than the 8-meter Würzburg of our present equipment.

For several years two or three of us have been intimately concerned with the design of large, movable parabolas for radio astronomy, and have served the government and other groups in these studies. Early in this report year (July 1957) an order was placed with the Blaw-Knox

Company of Pittsburgh for a 60-foot parabola on an equatorial mount, but construction has been delayed by government priorities, and erection is not expected until late autumn 1958. Somewhat larger parabolas (85 feet) based on the design developed here at the Department are being built by Blaw-Knox for the University of Michigan and for the National Radio Astronomy Observatory at Green Bank, West Virginia. During this year most of our efforts in connection with radio hydrogen have been devoted to systematic observations.

The 54-channel 21-cm radiometer described in last year's report gave gratifying performance for much of the year. With this instrument the whole spread of Doppler velocities in any one direction in the sky (up to 450 km/sec) is measured in a single observation lasting 4.8 minutes. No modifications were made of the electronics, most of the effort this year being expended on survey work and on diagnostic tests. In the previous report it was noted that, when the system is used on the sky, zero shifts of $\pm 1^\circ$ K can occur in a period of several hours. It appears that this effect is largely due to variable mismatch in the antenna itself. Empirical antenna adjustments are necessary to minimize the shift and slope of the zero line, a consequence of the rather large separation (six wavelengths) between antenna and mixer. In practice, the best method of overcoming the problem of zero level variability has proved to be the taking of check runs on parts of the sky known to contain little hydrogen. Such "cold sky" runs taken every few hours permit the determination of the zero level within $\pm 1^\circ$ K unless unusual atmospheric conditions such as snow or rain are prevailing.

The precision of single observations of 4.8 minutes' duration on each single channel of the 54-channel set was found to be $\pm 2.3^\circ$ K for each separate channel, and averages of three observations on the same

channel showed a statistical rms fluctuation of 1.5° K (P. E. 1.0° K). The same result was obtained for runs taken 2 months apart on cold areas of the sky. This value is in reasonable agreement with the input circuit equivalent noise temperature of around 1000° K indicated by the Bendix diode. Each channel is 12 kc wide to half-power points, and channels are centered on points 18.9 kc apart, corresponding to a Doppler shift of 4 km/sec from one channel to the next, each channel covering a hydrogen velocity spread of about $2\frac{1}{2}$ km/sec.

stant effectively broadens the filter bandwidth, thus diminishing the frequency resolution of the instrument, and long-period frequency variations affect the position of the local standard of rest and hence the accuracy of relative velocity measurements. Both effects were checked simultaneously by radiating an independent crystal oscillator harmonic close to 1420 mc/sec from the laboratory window toward the parabolic dish. When the local oscillators are properly adjusted, short-term frequency fluctuations appear to have negligible effect on the bandwidth, but the

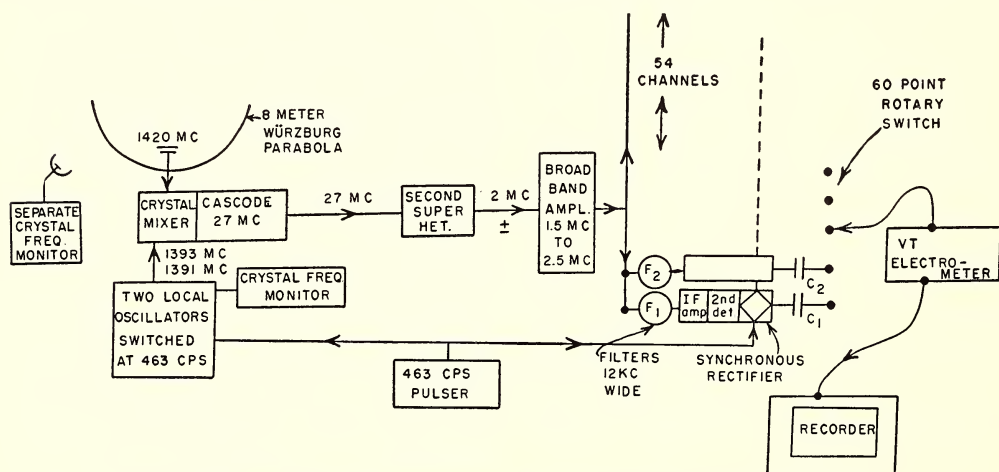


Fig. 2. Block diagram of 54-channel H-line receiver, CIW-DTM, 1957.

The over-all sensitivity of the instrument was measured by determining the peak temperature at several points in the sky by means of a Bendix noise diode, accepting the manufacturer's correction (0.70) for transit time at 1400 mc/sec. The hydrogen maximum at $l=147$, $b=0$ measured consistently $T=102^\circ \pm 2^\circ$; $l=50$, $b=0$ gave $T=99^\circ \pm 3^\circ$.

A source of concern had been the stability of the two local oscillators at approximately 1393 mc/sec, and particularly the H-frequency oscillator, on which velocity measurements depend. Both the short-term and the absolute frequency stability are of importance, for frequency variation in times short compared to one time con-

check has proved valuable as a routine measurement of absolute frequency, for the servo system that controls the H-frequency oscillator, though stable over periods of many days, does drift slowly with time. A desirable improvement, now under way, is the replacement of these self-excited, servo-controlled oscillators by crystal-controlled oscillators having the usual multiplier chains. A block diagram of the present equipment is shown in figure 2.

High-Latitude Survey

A particularly appropriate task for a multichannel receiver is the survey of large areas of sky, since the speed of

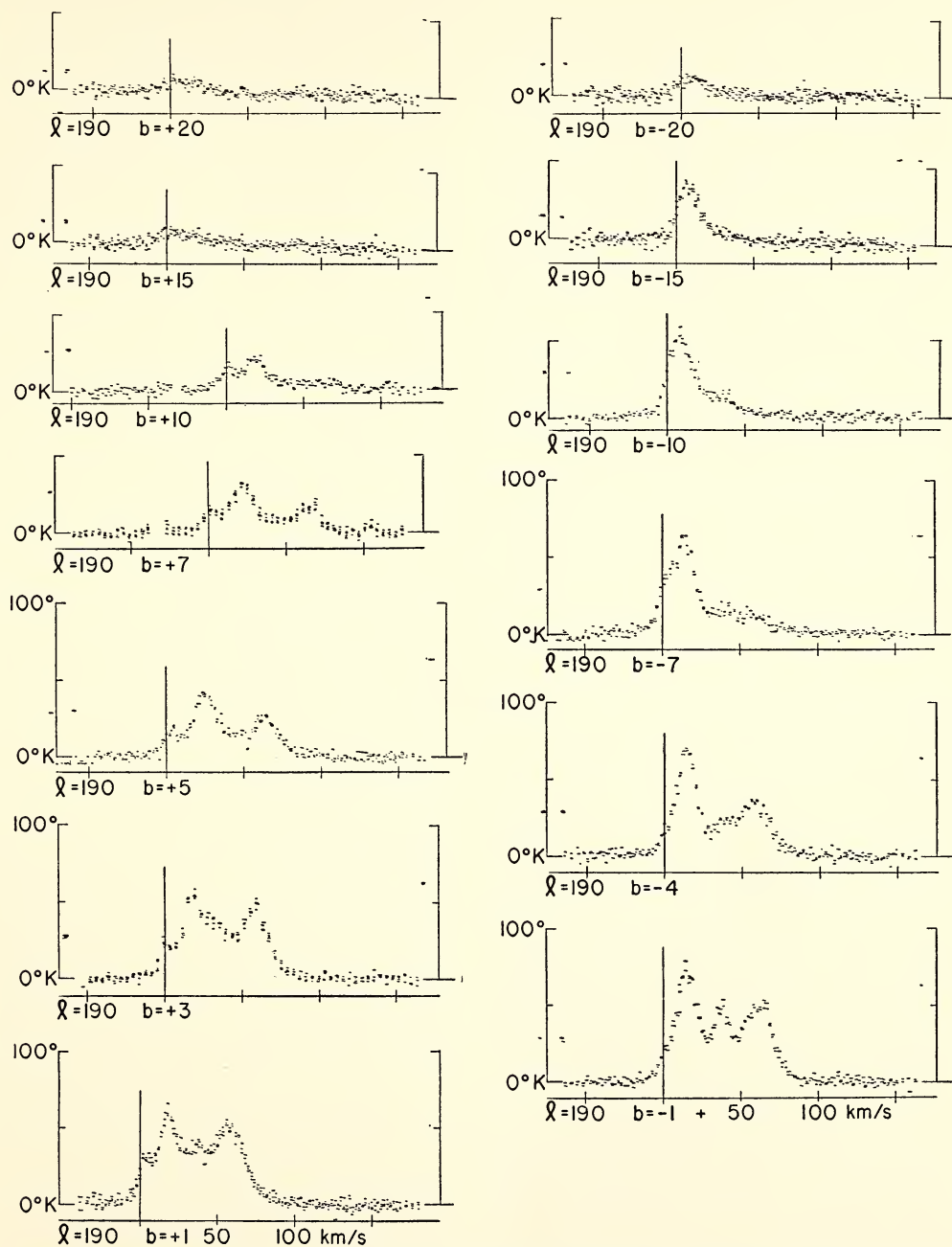


Fig. 3. H-line profiles taken with the multichannel recorder at a galactic longitude of 190° . The vertical line on each curve indicates the velocity of the local standard of rest.

gathering information is so much greater than for conventional scanning receivers. The first observing program, therefore, was a general survey of the entire visible sky at galactic longitudes from $\pm 20^\circ$ to the galactic poles in order to study the distribution and dynamics of the local interstellar gas. The observations were taken during the summer of 1957 and during the month of January 1958, using the 8-m Würzburg parabola. Each complete observation consisted of two sets of averages of one to three complete scans, interwoven in frequency. H-line profiles were observed at 10° longitude intervals along the $\pm 20^\circ$, $\pm 30^\circ$, and $\pm 40^\circ$ parallels of galactic latitude; at 20° intervals along the $\pm 50^\circ$ and $\pm 60^\circ$ parallels; at 40° intervals along the $\pm 70^\circ$ and $\pm 80^\circ$ parallels; and at the galactic poles. In addition, the $l=150^\circ$ – 330° meridian was observed at 10° latitude intervals, and scattered observations were taken near the galactic plane to correlate with the Leiden and Sydney surveys. Reduction of the profiles has proceeded (under the supervision of Dr. Helfer and Dr. Erickson, formerly Fellows of the Carnegie Institution at the Department) at the California Institute of Technology and at the Convair Scientific Research Laboratory, where a contour map has been constructed of integrated hydrogen intensity for the entire sky covered by the survey. In addition, contour maps have been constructed for each latitude strip showing isophotes of antenna temperature with relative velocity and galactic longitude as ordinate and abscissa.

Low-Latitude Galactic Survey

Since January 1958, most of the observing time of the 54-channel H-line receiver, in conjunction with the 8-m Würzburg, has been utilized for a survey of a strip 40° wide along the Milky Way. Our observed areas are at 010° , 013° , 017° , and on similar points for every 10° of longitude from $l=317^\circ$ through 0° to $l=227^\circ$ and have centered on a galactic plane slightly

tilted with respect to the old Lund equator. Thus, in the region to each side of $l=360^\circ$ we have centered our cross section on $b=1^\circ$; from $l=040^\circ$ to $l=110^\circ$ we have centered on $b=0$; and for $l=113^\circ$ to $l=227^\circ$ we have centered on $b=1^\circ$. Near the plane we observe at 2° intervals, increasing to 5° intervals beyond $b=10^\circ$. The antenna beam, to half-power points, is about $2^\circ \times 3^\circ$, elliptical in shape. Averages, usually of two sets of three scans each, interwoven in frequency, were taken as in the high-latitude survey, frequent checks of radiometer zero being made on "cold" sky. At the close of the report year, approximately half the survey had been completed, and a preliminary report, covering a single strip along the galactic equator, together with two meridian strips, had been prepared for the Paris Conference on Radio Astronomy (July 1958). A sample appears as figure 3, which shows the curves obtained along the $l=190^\circ$ meridian. A long vertical line on each curve indicates the frequency corresponding to zero velocity with respect to the local standard of rest. The usual convention is adopted, positive velocity corresponding to motion away from the observer. It is expected that the survey, when completed, will be useful in conjunction with optical observations for studies of the large-scale structure of the galaxy, in addition to providing a complete set of check points for detailed studies made with instruments of greater resolution. (B. F. B., W. C. E., J. W. F., H. L. H., H. E. T., M. A. T.)

THE EARTH'S CRUST

L. T. Aldrich, H. E. Tatel,⁴ M. A. Tuve, and G. W. Wetherill

The International Geophysical Year activities among our colleagues and friends the world over have served to stimulate and encourage extra efforts in our own areas of interest during 1957 and 1958.

Our geological age measurement pro-

⁴ Deceased November 15, 1957.

cedures, using selected minerals from special types of rock, have been thoroughly tested during the past several years. During these two years of the IGY particular efforts are being made to obtain samples from a wide range of locations. The previous age measurements from rubidium-strontium abundance ratios gave agreement with the potassium-argon age measurements when micas from granites and pegmatites were used. The resulting age scale was calibrated against concordant uranium-lead ages from some of the same pegmatites, and was also verified by careful laboratory determinations of the radioactive decay constants involved. Our range of sample rock types and locations has included suites of rocks from eastern Canada and both eastern and western United States as well as from Africa and Australia.

Other laboratories have now accepted our calibrations and adopted the same detailed procedures, and are rapidly and widely extending the geological provinces and type localities under study. We have likewise expanded our selected areas to include other parts of the United States and Canada, and we have also collected samples in parts of South America. As this report year closes we have staff members (summer 1958) in Europe and in the western United States collecting additional pertinent samples for age measurements. Further collaboration with colleagues in Africa and Australia is actively in progress, but some delays have been experienced in connection with the extension of our South American efforts.

The rock magnetism work, which has been such a conspicuous feature of our crustal studies for more than a decade, was largely discontinued this year when Dr. John Graham left us to join the staff of the Woods Hole Oceanographic Institution. Before he left, Dr. Graham brought to completion a remarkable series of field and laboratory studies. His direct demonstrations of the large effects of mag-

netostriktion on the remanent magnetization of rock samples, as measured after they have been uncovered by erosion and had been affected by various other geological processes, have challenged the validity of most of the spectacular claims made in the literature, especially by British workers, and have placed the burden of proof on those who claim to deduce magnetic directions in long-past geological epochs from measurements of this simple kind on rock samples. Clearly most rock magnetism observations are the fortuitous, although partly systematic, result of a long series of changes, both chemical and magnetostriktion, and any simple procedure for drawing "conclusions" as to the direction of the earth's magnetic field at the location of the rock sample during some period of the distant past appears to be thoroughly unjustified. Complex correction procedures, or some honest and unequivocal criteria for the retention or elimination of samples, may some day retrieve part of the body of rock magnetism data for discussion of such problems as the wandering of the geographic pole (shift of the crust relative to the axis of rotation) or for "continental drift," but discussions based on the kinds of data published to date by workers in several countries are, in our collective opinion here, deceptively romantic and misleading.

Rock magnetism data are still of considerable interest and value in connection with geological problems of much smaller scale, however, as in the working out of local or regional relationships. We have been interested in certain aspects of the magnetization, plastic flow, and subsequent chemical alteration and remagnetization of lava flows, for example. During 1957-1958 Mr. Donald Lindsley has been working on these problems at Johns Hopkins on a Carnegie scholarship under Professor Aaron Waters, and as the report year closes he is in the John Day Basin with our magnetometer equipment.

Among the geographical features of the

world there are relatively few elevated plateaus with large areas at consistently high elevations, compared, for example, with the many ranges of mountains. In studying the various large-scale geotectonic mechanisms that can be devised or imagined to account for continents and ocean basins, and in our thinking about the relative permanence of such great features, together with the long succession and specialized distribution of mountain-building activities, we have been attracted to the examination of these several high plateaus. They are of special interest in relation to our studies over the past decade using waves from large explosions for estimating the thickness of the "crust" of the earth. We have used explosion waves extensively in a search for "roots" of mountain chains. The idea that a mountain chain or plateau may be held at its higher elevation by having directly beneath it rocks less dense than the surrounding "mantle" rock is similar to the concept of the submerged ice "root" of an iceberg. The unexpectedly shallow crust we found in the Colorado plateau regions in 1955 with no indication of mountain "roots" made us very much interested in the high plateau of the Andes in South America.

The intense IGY activities in all countries stimulated us to undertake, as a major feature of our IGY participation, a seismic expedition to Peru, Bolivia, and Chile during the last half of 1957. It was carried out, supported in part by a generous grant from the National Science Foundation, as one of the two or three principal ways in which the Department has participated directly in the work of the International Geophysical Year. Another major aspect of our participation has been the five-station network across the magnetic equator, described elsewhere in this report.

SEISMIC STUDIES

Gravity measurements on continents and oceans, and over elevated plateaus and mountainous regions as well as sea-level

areas, show that the earth's crust is approximately in "floating equilibrium," evidently like rough ice on a pond. The high plateaus and mountainous regions show gravity values indicating that they are held up by the strength of the crustal surface to an extent that would support not more than 400 to 800 feet of rock. The rest of the elevation is due to the presence, somewhere under the plateaus, of a belt or thickness of material less dense, on the average, than the corresponding thickness of rocks beneath the low-lying plains. The traditional explanation for several decades has been that under mountain chains and high plateaus the lighter crustal rocks bulge downward into the heavier mantle rocks. Earthquake data have indicated the crustal rocks to be 30 to 35 km thick under continental areas of low elevation, and also have given some evidence of the expected downward bulge under the Alps and elsewhere. A rather sharp transition is observed in many localities between the bottom of the lighter "crustal rocks" (velocity 6 to 7 km/sec for compressional waves) and the heavier mantle rocks (velocity 8 km/sec); this transition is called the M discontinuity (the Mohorovičić discontinuity). Our expedition of 1955 to the Colorado plateau, however, showed the M discontinuity at about the same depths (29 to 33 km) under Arizona, New Mexico, and Utah, with average elevations of 6600 feet above sea level, as under the Chesapeake Bay region (31 to 34 km) and elsewhere at approximately sea-level elevations. This finding inclined us to accept an alternative hypothesis, namely, that the "mantle" rocks under the Colorado plateau, perhaps down to a depth of 150 km or more, might be of slightly (perhaps 1 per cent) lower density than the "mantle" rocks under the coastal plain and similar low-lying continental areas. This might be called the hypothesis of a "diffusely extended root." A situation like this, involving slight regional differences in the "mantle," would account for the elevation

of the mountainous regions just as satisfactorily as the older and highly specific picture of a downward-bulging "root" of lighter crustal rock. The M discontinuity under the Colorado plateau failed to show any such downward bulge of the lighter "crustal" rocks, at least where we were able to make explosion observations.

There are several other major plateau areas in the world, two of the most conspicuous being in the high Andes and in the Himalayas and adjacent, rather inaccessible, regions. For about five years we had been considering the attractive possibility of making observations in the Andes, inasmuch as in southern Peru and in northern Chile there are several sizable open-pit copper mines in which charges as large as 30 or 50 tons of explosive are shot several times each week. During 1957 the shooting schedule reached a new high level of activity. We undertook, then, on our IGY expedition to measure the waves from these explosions, as they propagated along lines parallel to the Andes and also as they passed across and beneath the towering ranges of the Andes and the high (14,000 feet) plateau of Peru, Chile, and Bolivia.

Our group of eight men and six trucks left Washington in July 1957 and assembled in Lima, Peru, in mid-August. During the next three months we made observations and recordings at more than 200 sites in Peru, Bolivia, and Chile, each site being carefully selected for low level of local seismic "noise" or ground unrest. The selection of a specific site in a desired locality often required many miles of difficult travel and noise level tests at many possible rock outcrop sites. Radio timing techniques were used on nearly 50 large explosions while our observers and their trucks, traveling in pairs, were deep in the mountains 80 to 500 km away. Most of these explosions were at the Toquepala Mine of the Southern Peru Copper Corporation, about 60 miles northwest of Tacna, Peru. During the latter part of the expedition the observed explosions were

at the Chuquicamata Mine of the Chile Exploration Company, about 125 miles northeast of Antofagasta, Chile. Both these mines are on the western edge of the plateau, at elevations of about 10,000 feet. In Peru we were greatly assisted by Dr. J. Broggi and staff members of his Instituto Geofísico de Huancayo (offices in Lima); one of the staff members participated in our observations in the Lake Titicaca region. In Chile we were joined by Fr. G. Saa, S. J., of San Luis College, Antofagasta, and Dr. C. Lomnitz, Director of the Centro de Geofísica, University of Chile, Santiago. These two scientists helped us in our measurements on the M discontinuity in the regions inland from Antofagasta.

Although our records apparently show the M discontinuity in both Peru and Chile, for wave paths running along the flank of the plateau, at medium elevations, we cannot deny our disappointment in finding that we could not observe the waves much beyond 200 km in directions across the mountains and across the plateau, and that we could find no evidence for locating the depth of the M discontinuity in these directions.

We had revised our seismic equipment for this expedition, building new seismometers (period 5 cycles per second) with higher output, to give signals well above the electronic noise level of the amplifiers. Our new equipment would measure vertical amplitudes of ground motion as low as $\frac{1}{10}$ or even $\frac{1}{100}$ angstrom unit (10^{-9} cm), in the frequency range 5 to 25 cycles per second. In North America very few sites have ever been found with so low a level of ground unrest. When we found the attenuation of the waves across the "altiplano" so extreme, we made special efforts to select quiet sites, and at many of our observing stations a wave arrival as small as $\frac{1}{2}$ angstrom in amplitude would have been definitely identified, yet in the range from about 230 km out to about 550 km, across the plateau, no wave arrivals were found. In Peru and Bolivia we occupied

more than 70 such frustrating sites, from north of Cuzco to the east of Oruro, Bolivia, a few miles from Cochabamba. In our extensive studies in eastern, central, and western United States and in the Yukon-Alaska regions we have never encountered anything even resembling such extreme attenuation of explosion waves. The Andes are clearly different from other mountains we have studied.

Meanwhile, in the course of numerous truck repair episodes involving trips down from the plateau, large-amplitude wave returns were finally discovered for paths in Peru to the northwest of Toquepala, along the flank of the high plateau, and the anomalous attenuation was not observed along these paths. These large-amplitude waves were very much like those recorded in the Rocky Mountains, with normal first arrivals out to 200 km and beyond, and strong second arrivals in the region of 200 to 230 km. In the Rockies the strong second arrivals are observed at 100 to 150 km from the shots. On the flank of the Andes, however, the terrain is too rough to place observers at each of the desired distances and thus set precise limits on the range of distances in which the "reflections" are observed. The velocities appeared to be normal (about 6 and about 8 km/sec) for both crust and mantle regions, and the M discontinuity was thus indicated to be in the range of perhaps 44 to 48 km depth, on the basis of the strong second arrivals at 200 km and the first arrivals at farther distances, and the possible existence of a "hidden" layer of intermediate velocity rock (7 km/sec) just above the M discontinuity. Our first impression was that of a normal depth around 34 (or 36) km, based on the two usual rock types, 6 and 8 km/sec; this is revised to an alternate estimate of about 46 km depth if there is an intermediate layer above the M discontinuity. These sketchy observations can hardly be considered a satisfactory measure of the depth of the crust, because the compressional waves through the mantle

(P_n arrivals) could not be observed out to the necessary distances of 300 to 400 km to give a good measure of the deeper velocities. Along the flank of the Andes this was due to the topography of the country, which limited our access to desired locations.

In view of the great effort expended in the Peruvian mountains in obtaining these rather inconclusive measurements, the very marked attenuation of the waves across the plateau into Bolivia, and the shortness of time, the expedition moved to Chile in early October 1957. Observing sites were again established on the high plateau (14,000 feet) in Chile and Bolivia, to the north and east of Chuquicamata, and again the same very marked attenuation was observed as in Peru and Bolivia, contrary to our experience in North America. Again, however, search for large-amplitude wave returns from a deep reflecting zone, as from the M discontinuity, was successful along the flank of the mountains, this time to the south. The intense wave returns (second arrivals) were first observed here at 220 km from the shot, and beyond, indicating a depth of about 46 km to the M discontinuity, if only the two rock types are present (6 and 8 km/sec). In Chile there was a faint indication of a second "layer" of intermediate velocity near 7 km/sec, and if this is actually present the depth to the M discontinuity is about 56 km, much greater than any we have hitherto observed.

Neither the data from Peru-Bolivia nor those from Chile-Bolivia can be considered satisfactory measures of the crustal structure in those regions, or even as definitive measures of the existence and depth of the M discontinuity. The large-amplitude second arrivals observed in both regions, however, surely were similar to those we have observed in North America, although they were found at definitely much greater distances from the explosion points than any in our previous experience. The observations obtained in Peru, Bolivia, and Chile are shown in figures 4, 5, and 6.



Fig. 4. Seismic observations on the waves from explosions were made by the Carnegie-IGY Andes Expedition, 1957, at intervals out to several hundred kilometers along lines radiating out from the large open-pit copper mines at Toquepala, Peru, and Chuquicamata, Chile, approximately as shown. The high ranges of the Andes lie just to the east and north of Arequipa and the two mining centers, and the high plateau extends eastward beyond La Paz.

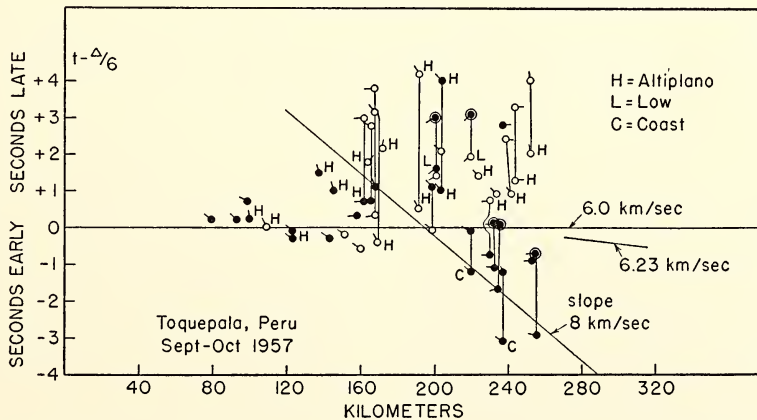


Fig. 5. Arrival times of explosion waves from the mine at Toquepala, Peru, show no evidence of any layer of velocity intermediate between the crust (6 km/sec) and the mantle (8 km/sec), and give only a rough indication of the depth to the M discontinuity (36 km if no intermediate layer). Waves in and under the high Andes were too strongly attenuated to be observed.

Four of the special trucks the Department bought and equipped for the Andes expedition were left in South America, in the hope that arrangements might be made by friends and colleagues in South American institutions to continue and extend our seismic and gravity measurements. Reductions of the IGY appropriations by the government of Peru have restricted the opportunities for collaboration there, but in Chile a whole series of measurements is being supported by the University of Chile. Dr. Cinna Lomnitz, Chief of the

and Chile Exploration Company); E. M. Tittman and C. Pollock (New York officers of the American Smelting and Refining Company and the Southern Peru Copper Company); Charles McGraw, Lima (Manager of the Marcona Mining Company); and O. C. Laird, Caracas (officer of the Orinoco Mining Company); and Warren T. Smith and Marion Robinson, local officers of the Southern Peru Copper Company at Toquepala. In addition, the officials of the International Geophysical Year in Peru, Bolivia, Chile,

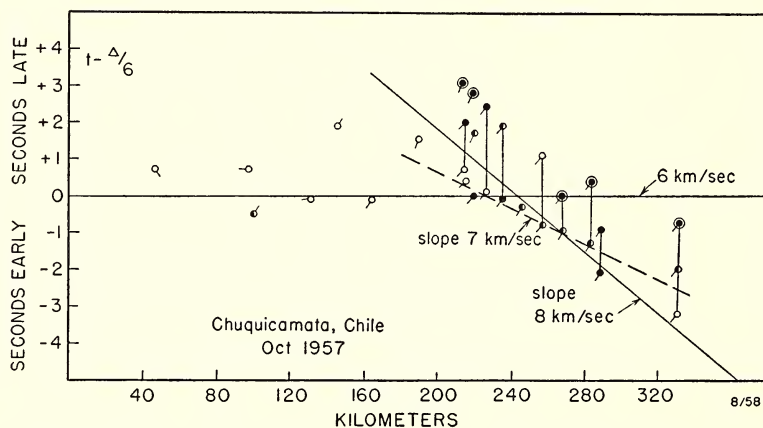


Fig. 6. If the intermediate layer indicated (7 km/sec) is actually present, the M discontinuity lies at 56-km depth to the south of Chuquicamata (tabs on points show direction of wave travel); for the simpler case (6 km/sec crust overlying 8 km/sec mantle) the calculated depth is nearer 46 km. Again the waves were strongly attenuated across the high Andes.

new Center for Geophysics at that University (in Santiago), has already made measurements across the Andes escarpment at different latitudes, using our Worden gravity meter, and as this report is written (July 1958) he and his colleagues are beginning a further series of observations on the explosion waves from Chuquicamata using our seismic equipment.

The Institution acknowledges with pleasure the cordial participation of the officials of the several mining companies concerned in the arrangements for these explosion wave observations: C. E. Weed, Charles M. Brinkerhoff, Thomas A. Campbell, and D. M. Dunbar (New York officers of the Anaconda Copper Company

Argentina, and Venezuela were most helpful and cordial in making local arrangements in their respective countries. We received permission, for example, to use our two-way radios in each of these countries.

We owe the local officials at Chuquicamata, especially Robert C. Becker, Deputy Manager, W. E. Rudolph, W. H. Swayne, and John Kent, and members of their geology and engineering staffs, a measure of gratitude that is very deeply and personally felt. We had been experiencing difficulties in reaching desired distances in the mountains, along selected directions from the shots, owing to various mechanical failures of our four-wheel-drive

heavy-duty trucks. When we were so distressed by the prospect of further breakdowns at inaccessible points, far from any normal roads or traffic, that we were ready to abandon an important sector, these courageous officials offered to send a retriever expedition from the mine, if necessary, and gave us the courage to proceed. Part of our resulting efforts were successful, but one of our vehicles lost a rear axle deep in the mountains near the Salar de Atacama, and it required two successive four-man expeditions from the mine, over a period of two weeks, to retrieve our truck, which was finally brought out by a southern route, several hundred miles from the mine. The magnificent hospitality and helpfulness of this action, coming as it did when the members of our expedition were tired out and due to return, will never be forgotten.

MINERAL AGE MEASUREMENTS

*L. T. Aldrich, G. W. Wetherill, G. R. Tilton,⁵
and G. L. Davis⁵*

In previous annual reports of this group, independent mineral ages obtained from cogenetic minerals from the same rocks have been used to check the consistency and agreement of ages as determined from the measured ratio of daughter to parent isotopes from the decay of isotopes of uranium, thorium, rubidium, and potassium.

The results of these measurements may be summarized briefly as follows: (1) the fact, first demonstrated by Wasserburg, Hayden, and Jensen, that the two uranium-lead ages of pegmatitic uraninites almost always agree (are concordant) has been shown for several additional locations; (2) rubidium-strontium ages of micas and potassium feldspars agree with the concordant uranium-lead ages of cogenetic uraninites within 5 per cent; (3) potassium-argon ages of mica agree with uraninite ages within 10 per cent, and, though Wasserburg, Hayden, and Jensen

have shown that pegmatitic feldspars seem to lose an amount of argon nearly proportional to the time since they were formed, this property is not displayed by feldspars generally; (4) the rubidium-strontium and the potassium-argon ages of micas from unaltered rocks usually agree with each other, and when they do not the disagreement is always in the direction that indicates some leakage of argon from the mica; (5) micas from metamorphosed rocks display a variable and as yet not fully understood pattern of ages; (6) it has been further shown that the uranium-lead ages of monazite, zircon, and columbite-tantalite are not predictably concordant.

It could have been predicted from last year's report that this year's work would be primarily concerned with two problems. The investigation of geographical patterns of the ages of the Precambrian rocks of North America and the investigation of the ages of minerals involved in metamorphic processes have both been continued. Progress on the geographical patterns has not been limited by our own capacity for measurements. The data obtained at the Lamont Geological Observatory and the Geology Departments of the University of Minnesota and the Massachusetts Institute of Technology, examined together with our own, show conclusively the broad outlines of major periods of mineral formation for the parts of the continent that have now been measured. These patterns are shown on the map of figure 7. The points with stubs attached are data obtained at other laboratories.

A large area with rocks of ages exceeding 2500 million years extends from western Quebec through Ontario to eastern Saskatchewan and northern Minnesota and reappears in Montana and Wyoming. Another large area with rocks of ages close to 1350 million years lies in western United States, and rocks of this age have now been found in Missouri, Wisconsin, Michigan, and Ontario, so that this period also appears to have been one in which

⁵ Geophysical Laboratory, C. I. W.

processes of mineral formation occurred over very widespread areas in North America. It is of some interest to note that no occurrence of rocks of this age has yet been found outside this continent. A final large region with rocks of similar age extends from northern Quebec south into Ontario, New York, New Jersey, Virginia, and North Carolina. All these rocks were formed close to 1000 million years ago.

mineral formation in the Baltimore area during the period 300 to 350 million years ago had been found by Wasserburg, Lipson, and Pettijohn. Because the Baltimore gneiss is a metamorphic rock these mica ages probably date the metamorphism, but give no information about the premetamorphic history of the rock. New mineral age measurements indicate the possibility of two periods of mineral formation. Dur-

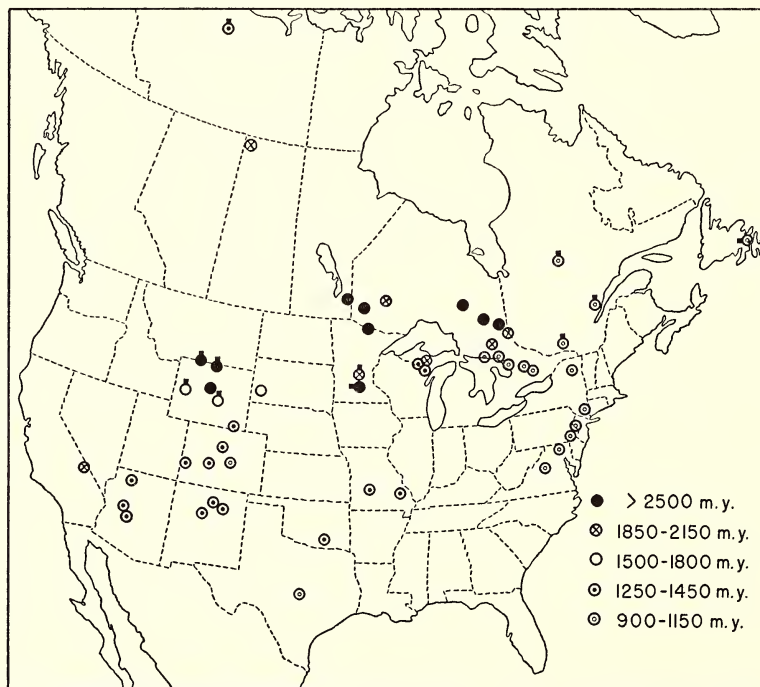


Fig. 7. Reliably dated localities in North America.

Regions near the boundaries of these areas often contain rocks of different ages, and the patterns of the ages obtained from different minerals in the same rocks indicate a multiple history of mineral formation. Such areas appear notably in the Sudbury region of Ontario and the upper peninsula of Michigan, and may well be found elsewhere as additional data are obtained.

The major part of the study of metamorphic rocks has been the investigation of the Precambrian rocks of the central and southern Appalachians. Evidence of

ing one of them (1000 to 1100 million years ago), zircon and probably potassium feldspar were formed; in the second (300 to 350 million years ago), the mica of the gneiss was formed. Measurements of other Appalachian gneisses give mica ages agreeing more closely with the zircon ages, a further indication of the Precambrian origin of the metamorphosed rocks. Again, the power of the attack on geologic problems by all methods of age determination on all available minerals has been demonstrated.

In another study, micas from many of

the zones of the regionally metamorphosed area in Iron County, Michigan, have been measured. This area has been studied intensively by Dr. H. James and his colleagues of the United States Geological Survey, with whose guidance the suite of rocks for our study was obtained. In summary, the ages of the mica from the sillimanite, garnet, and biotite zones are all the same within the error of measurement (5 per cent). Further, this age is that of the mica from fresh granite thought to be the energy source for the regional metamorphosis.

The Grenville Orogeny in the Appalachian Region

The existence of rocks between 900 and 1100 million years old in the southern portion of the Canadian Shield known as the Grenville Subprovince has been recognized for many years. Until recently these rocks have been found only in a relatively small area in Quebec and Ontario. It had been suspected for some time that the igneous and metamorphic rocks of the Adirondack Mountains in New York represented a southern extension of this subprovince. It had also been suspected that the entire complex of granites, pegmatites, gneisses, and metasediments represented the deeply eroded core of an ancient mountain system trending in a northeast-southwest direction through Ontario, Quebec, and New York. A few years ago Tilton measured a sample of zircon from Natural Bridge, New York, in the Adirondacks and found an age very similar to ages in the Grenville of Canada. This finding strengthened belief in the idea that the Adirondacks are a southern extension of the Grenville rocks. If these rocks really were formed during an orogenic (mountain-building) epoch, however, it would be expected that this mountain chain was linear and extended for thousands of miles, as do more modern mountain chains. Therefore, it should be expected that more 1000-million-year-old rocks exist farther

along the axis of the hypothetical mountain system.

About two years ago workers at Columbia University showed that rocks in the New Jersey and Hudson Highlands, near New York City, were Precambrian in age. Further work of our group (reported in Year Book 56) confirmed this discovery and showed that their age was approximately the same as that of the Grenville rocks farther north. During the past year the measurements have been extended to the south, and the existence of rocks of "Grenville age" at least as far south as Virginia has been demonstrated.

The principal difficulty encountered in measuring the ages of the Precambrian rocks in the Appalachian region is that all of them have been, to a greater or lesser extent, involved in the tectonic movements and metamorphic events of the Paleozoic Appalachian orogeny. In so far as they have been recrystallized during this more recent orogeny, the record of their original age has been destroyed. Thus the mica in most of the metamorphic rocks in the Appalachian region has an age of around 300 million years, and there is no way of knowing whether the rock is only slightly older than 300 million years or is actually of Precambrian age. As is shown in tables 1 and 2, however, some rocks have been found that still bear evidence of their Precambrian age.

The group of rocks listed in table 1 all contain zircons of Grenville age together with micas probably 1000 million years old that have suffered some alteration during the Appalachian orogeny. All these micas, however, are definitely pre-Appalachian in age, and the simplest interpretation of the results is that both the mica and the zircon were formed during the Grenville orogeny, and that the gneisses represent a further extension of the Grenville gneisses.

Measurements on samples of the Baltimore gneiss are given in table 3. The Baltimore gneiss underlies all the metasedimentary rocks in the vicinity of Baltimore and has generally been regarded as

TABLE 1. Mineral Ages of Precambrian Gneisses

Location	Mineral	Age, million years					
		U^{238}	U^{235}	Pb^{207}	Th^{232}	Rb^{87}	K^{40}
		Pb^{206}	Pb^{207}	Pb^{206}	Pb^{208}	Sr^{87}	A^{40}
Bear Mountain, N. Y.	Zircon	1140	1150	1170	1030		
Canada Hill gneiss	Biotite					900	780
Storm King granite	Zircon	960	990	1060	850		
	Biotite					940	
Shenandoah National Park, Virginia	Zircon	1070	1100	1150	1110		
Mary's Rock Tunnel gneiss	Biotite					890	800
Hibernia, N. J. gneiss (dark)	Biotite					920	
gneiss (light)	Biotite					840	

TABLE 2. Mineral Ages from the Baltimore Gneiss

Location	Mineral	Age, million years					
		U^{238}	U^{235}	Pb^{207}	Th^{232}	Rb^{87}	K^{40}
		Pb^{206}	Pb^{207}	Pb^{206}	Pb^{208}	Sr^{87}	A^{40}
Baltimore area	Zircon	1040	1070	1120	940		
Towson dome	Biotite					305	339
	Microcline						309
Phoenix dome	Zircon	960	1020	1120	1100		
	Biotite					310	
	Microcline					1190	
	Microcline					1130	
Woodstock dome	Biotite					310	
Philadelphia area	Zircon	1010	1045	1120	950		
Spring Mill, Pa.	Biotite					390	550

TABLE 3. Ages of Metamorphic and Igneous Rocks in the Washington-Baltimore Area

Sample	Mineral	Rb-Sr	K-A	U^{238}	U^{235}	Pb^{207}	Th^{232}
				Pb^{206}	Pb^{207}	Pb^{206}	Pb^{208}
Kensington gneiss							
Sample A	Biotite	305	380				
	Zircon			370	395	550	
Sample B	Biotite		350				
	Zircon			400	420	510	350
Woodstock granite	Biotite	314					
Baltimore gneiss							
Towson dome	Biotite	305	339				
Woodstock dome	Biotite	322					
Phoenix dome	Biotite	310					

of Precambrian age. Wasserburg, Lipson, and Pettijohn have recently shown, however, that the K-A ages of the metamorphic mica in these overlying sediments, as well as that in the Baltimore gneiss itself, are Paleozoic. Thus the metamorphism of the gneiss was Paleozoic, and its Precambrian age cannot be inferred from its degree of metamorphism. Consequently, the question of the age of the gneiss was reopened. As is shown in table 2, zircon and feldspar of Grenville age have now been found in the gneiss. Petrographic examination of the rock indicates that it is improbable that the feldspar is of detrital origin. In agreement with the findings of Wasserburg et al., the mica appears to have been recrystallized during the metamorphism. The Rb-Sr ages of micas from the Baltimore gneiss near Baltimore are compared with those of the intrusive Woodstock granite and the concordant Kensington granite gneiss in table 3. All the ages are in agreement within their experimental errors, and indicate that the metamorphism in this area took place 310 ± 10 million years ago. The Baltimore gneiss at Philadelphia occupies a similar stratigraphic position, but the correlation of the sediments and gneiss between the two areas is obscure. The mica from the Philadelphia sample does not seem to have been so completely recrystallized as that in the Baltimore samples. Again the zircons indicate a Grenville age.

It is interesting to note that the K-A age of the mica from the Philadelphia sample is much greater than its Rb-Sr age. The same phenomenon is observed to a lesser degree in the Kensington granite gneiss and the Towson dome samples. This effect has previously been found in metamorphic rocks near Sudbury, Ontario, as was reported by this group in the previous annual report and was also found by the geochronology group at Massachusetts Institute of Technology. It seems to be peculiar to metamorphic rocks, since, in the fifty or more cases measured in this laboratory where Rb-Sr and K-A ages

have been compared on the same mica sample from igneous rocks, the K-A age is invariably younger. It may be that during the recrystallization of the metamorphic rocks the old radiogenic argon is not completely excluded from the mica.

Therefore, it appears that the gneisses listed in tables 1 and 2 are to be grouped with the Grenville rocks farther north. As is indicated in figure 7, this grouping extends the Grenville orogeny a considerable distance to the south of the original area.

Recent measurements reported by the Massachusetts Institute of Technology and the University of Minnesota groups now extend the 1000-million-year-old rocks up into northern Quebec and Newfoundland, thus fulfilling the expectation that a long linear belt of these rocks would be found.

Age Patterns in Zones of Regional Metamorphism

The rocks from the metamorphosed zones in Iron County, Michigan, are all part of the Michigamme Formation, which is a slate placed in the middle Precambrian section. The Mary Lake granite intrudes the Michigamme slate, and its relationship to the metamorphic zones of the slate is such that it is indicated as the source of energy for the regional metamorphism. The mineral age measurements obtained on mica from this granite and the various metamorphic zones are given in table 4. All the Rb-Sr ages agree within the error of measurement. The K-A ages are in general agreement with the Rb-Sr ages but consistently lower. The muscovite in the sillimanite zone is doubtless secondary, and its K-A age is indicative of this fact.

It is concluded that the mineral age measurements are not in disagreement with the field evidence of the regional metamorphic pattern. The pegmatites in neighboring Dickinson County intrude a pre-Huronian granitic series, and it had previously been assumed that the metamorphism from the granitic intrusion and

the intrusion of the pegmatites were simultaneous. These measurements show conclusively that at least 300 million years separated the two events.

obtained at our laboratory are given by age in table 5. The 1000-million-year-old rocks have been discussed more completely above. Additional locations of 2600-mil-

TABLE 4. Ages of Micas from Northern Michigan

Source	Mineral	Age, million years	
		K-A	Rb-Sr
Iron County, metamorphic zones			
Mary Lake granite	Biotite	1330	1390
Sillimanite Zone (Peavy Falls)	Biotite	1240	1390
Sillimanite Zone (Peavy Falls)	Muscovite	1140	
Garnet Zone (Horseshoe Rapids)	Biotite	1100	1380
Biotite Zone (Cedar Falls)	Biotite	1280	1420
Republic, Marquette County	Pegmatite, muscovite	1760	1830
Felch, Dickinson	Pegmatite, feldspar		1760
Felch, Dickinson	Pegmatite, muscovite	1630	1720

TABLE 5. Ages of Micas

Source	Mineral	Age, million years	
		K-A	Rb-Sr
2600 m.y. ages			
Kirkland Lake, Ont.	Granite	2530	2600
Timmins, Ont.	Granite	2520	2470
Hearst, Ont.	Pegmatite	2595	2600
East of Kenora, Ont.*	Granite		2550
Winnipeg River, Man.	Pegmatite	2150	2640
International Falls, Minn.*	Gneiss inclusion	2650	2610
Bonneville, Wyo.	Pegmatite	2256	2420
1350 m.y. ages (1957-58)			
Mary Lake, Iron Co., Mich.	Granite	1330	1390
Menominee, Wisc.	Granite	1230	1370
Frederickstown, Mo.	Pegmatite	1405	1450
Decaturville, Mo.	Pegmatite		1450
Troy, Okla.	Granite		1360
Miscellaneous (1957-58)			
Sioux Lookout, Ont.	Gneiss		2190
Wichita Mts., Okla.	Granite	460	500
Death Valley, Calif.†	Pegmatite	1660	1725
Dayton Bend, N. C.	Gneiss		370

* Analyzed 1957-1958.

† In collaboration with G. J. Wasserburg.

This year's work has increased the information available on the geographic extent of the 2600-million-, 1350-million-, and 1000-million-year orogenies by almost a factor of 2. The data for the map of figure 7 for the first two groups which have been

lion-year rocks measured this year are shown with our previous measurements in the table. The work of P. W. Gast at the Lamont Geological Observatory has shown the general occurrence of rocks of this age both in the Winnipeg River area in west-

ern Manitoba and in the Big Horn Mountains of Wyoming. Goldich, Baadsgaard, and Nier at the University of Minnesota are responsible for most of the data on Minnesota rocks. Hurley, Fairbairn, and Pinson have provided most of the ages in Ontario between Sudbury and Sioux Lookout. The western Quebec data are from the University of Toronto.

Data on eleven 1350-million-year-old granitic rocks in western United States were summarized last year. It is seen in the tables that orogenies in this period left their imprint in Oklahoma, Missouri, Wisconsin, Michigan, and southern Ontario. The long-known uranium ore of this age at Great Bear Lake, N.W.T., Canada, is further demonstration of the common occurrence of minerals of this age. The older age of the Death Valley sample, measured in collaboration with Wasserburg, indicates that the area included in this orogeny is bounded by older Precambrian rocks to the west.

Additional rocks and minerals of the Cutler batholith that have been analyzed are shown in table 6, together with those previously obtained. That there were two important periods in the history of this rock unit seems obvious, but there remain the little-understood discrepancies in the K-A and Rb-Sr ages of the same mineral, which will preclude any complete interpretation of the data until the effects of alteration on mineral ages are better understood.

The Department of Terrestrial Magnetism members of this mineral age group took part in the Carnegie Andes Seismic

Expedition during July–October 1957, and collected a small number of rocks in Peru. The rocks analyzed had been mapped as pre-Cretaceous, and from the data of table 7 (a middle-Cretaceous age of 100

TABLE 6. Summary of Age Determination Work on the Cutler Batholith

Source	Mineral	Age, million years	
		K-A	Rb-Sr
Large pegmatite	Muscovite	1390	1750
	Feldspar	1120	1760
Mica schist in contact with above			1240
Small pegmatite	Muscovite	1370	1700
Granite 1	Biotite		1310
	Muscovite		1430
Granite 2	Biotite	1330	1325

TABLE 7. Ages of Peruvian Micas

Source	Age, million years	
	K-A	Rb-Sr
Macchu Picchu, Dept. Cusco, Peru		200±20
Mollenda, Dept. Arequipa, Peru	410±30	400±40
Atica, Dept. Arequipa, Peru	330	300±100

million years was measured also on a pegmatite from Pala, California) one would conclude first that they are indeed pre-Cretaceous as mapped and secondly that they are not Precambrian. Other samples obtained from Chile and Brazil will be analyzed in the coming year.

THEORETICAL AND STATISTICAL GEOPHYSICS

S. E. Forbush

EQUATORIAL ELECTROJET

As was stated in last year's report the investigation of the equatorial electrojet is being carried out as part of the United States program for the International Geophysical Year (IGY) through grants ap-

proved by the IGY Panel on Geomagnetism. The four Askania variographs loaned to the Department of Terrestrial Magnetism through the cooperation of the U. S. Coast and Geodetic Survey are being operated on the west coast of Peru at

Talara, Chiclayo, Chimbote, and Yauca, which extend from geographic latitude 4° N to 15° S.

These four temporary magnetic observatories are operated and maintained through the cooperation of the John A. Fleming Observatory of the Instituto Geofísico de Huancayo, which also provides magnetic variation data from Huancayo. The Department of Terrestrial Magnetism has cooperated with the Instituto Geofísico de Huancayo in the establishment of a permanent magnetic observatory for the University of Arequipa (latitude 17° S) by providing a la Cour vertical intensity variometer. This observatory is expected to be in operation by July 1958, when its records will become available for investigation of electrojet effects.

Owing to delay in procurement and to serious defects in instruments rather few simultaneous and complete daily records have been obtained at the four variograph stations. Consequently the few magnetic-storm sudden commencements which occurred near midday were too inadequately recorded to indicate whether they were influenced by electrojet effects. Most of the instrumental defects have now been corrected, and it is expected that by July 1958 satisfactory records will be available from the four Askania variographs. These, with records from Huancayo and the new Arequipa Observatory, should provide records from a total of six stations.

From results obtained on the preliminary survey mentioned in last year's report the maximum diurnal variation in H was found within 1° in latitude from Huancayo (12° S) in accord with results obtained by A. A. Giesecke, Jr., Director of the Instituto Geofísico de Huancayo, with QHM's in 1949. This result is also confirmed by the fact that the diurnal variation in Z from the 1957 survey changes sign at a latitude within 1° of Huancayo. The maximum diurnal variation amplitude in Z occurred at about latitude 16° S and the minimum near 8° or 9° S.

The latitudinal distance of 500 miles be-

tween these extremes provides a rough estimate of the width of the electrojet band. The variation with latitude of the amplitude of the diurnal variation of Z indicates the importance of the return currents (from the main eastward current band) which must flow westward, north and south of the electrojet.

To effect reliable estimates of the width and height of the electrojet will require derivation of the electric current system for the electrojet field, after deducting the field of the "normal" S_q diurnal variation estimated from observatory data at locations free from the influence of the electrojet.

A preliminary examination of records indicates that during disturbed periods many of the fluctuations in Z have the largest absolute magnitude at the latitude where the largest amplitude of S_q in Z was observed. The sign of these fluctuations in Z is opposite at these two latitudes, thus indicating electrojet effects.

COSMIC-RAY INVESTIGATIONS

Cosmic-ray variations and solar activity. During the period 1937 to 1957 two entire cycles of solar activity were completed, and in 1957 there occurred the largest sunspot numbers on record. Figure 8 shows the annual means of sunspot numbers and those for cosmic-ray intensity from Huancayo and from Cheltenham (Fredericksburg) for this interval. The years of maximum cosmic-ray intensity occur near those for minimum sunspot numbers. Moreover, the minima of cosmic-ray intensity in 1947 and 1957 were lower than in 1937, a finding in accord with the fact that sunspot numbers were greater in 1947 and 1957 than in 1937. The decrease in cosmic-ray intensity between 1955 and 1957 evidently lags a year or so behind the increase in sunspot numbers in that period. Figure 9 shows that the decrease in cosmic-ray intensity at Huancayo between 1954 and 1957 also lagged behind the decrease recorded by D. C. Rose with a neutron monitor at

Ottawa and behind that observed by H. V. Neher in balloons at Thule. Near sunspot minimum in 1954 Neher found, near the top of the atmosphere at Thule, that there was no evidence for the exclusion of low-energy primary particles—at least not for protons with energy 50 Mev or more. When solar activity increased after 1954 the primaries near the low-energy part of the cosmic-ray spectrum were first excluded; later as the solar activity cycle pro-

cesses, a barrier appears to be created which effectively prevents primary particles from our galaxy from reaching the earth. This barrier most likely comprises plasma clouds with magnetic fields ejected from the sun. Such individual plasma clouds must account for magnetic storms and, through magnetohydrodynamic effects, for large sudden decreases in cosmic-ray intensity that sometimes occur during magnetic storms. Analysis of IGY cosmic-ray

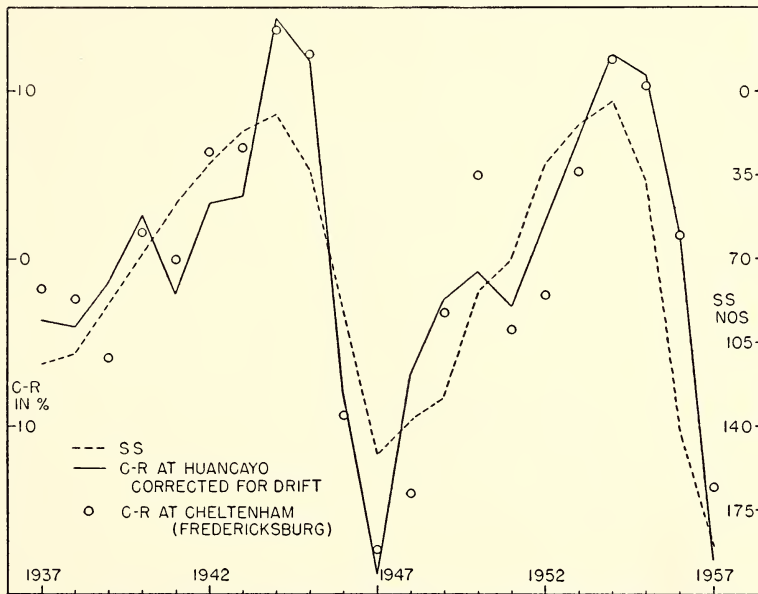


Fig. 8. Annual means cosmic-ray intensity (C-R) and sunspot numbers (SS).

gressed toward a maximum, in 1957, primary particles with greater and greater energy appeared to be excluded. This exclusion accounts for most of the decrease in high latitudes, as in figure 9 for Thule and Ottawa. The decrease in intensity at Huancaayo which started in 1956 indicates a reduction in the number of primary protons even for energies above at least 15 bev. Neher's results at Thule showed there was, in 1954, no "knee" in the variation of intensity with latitude, near the top of the atmosphere. The work of several investigators shows that by 1957 the "knee" was near latitude 48° geomagnetic.

Thus with increasing solar activity a

data from several latitudes should provide information on the change in energy spectrum of primary particles during the solar cycle and also during transient decreases which should lead to a better understanding of the mechanism responsible for these changes in cosmic-ray intensity. This in turn is certain to reveal important changes in electromagnetic conditions in the solar system that could not be revealed in any other way.

The change, with solar cycle, of the variability of cosmic-ray intensity is shown in figure 10 by the yearly pooled standard deviation of daily means from monthly means. Near the sunspot minima in 1944

and 1954 the standard deviations were also minimal. These minimal values are at about the noise level of the instrument. In figure 10 it is seen that the standard deviation is decidedly larger in 1957 than in 1937, in accord with the much larger sunspot maximum in 1957 than in 1937.

sure have been completed for Huancayo and Fredericksburg through April 1958. From the records scaled at Christchurch the reduction of daily means has been effected through June 1957. To cooperate in the United States program for cosmic-ray research in the International Geophysical

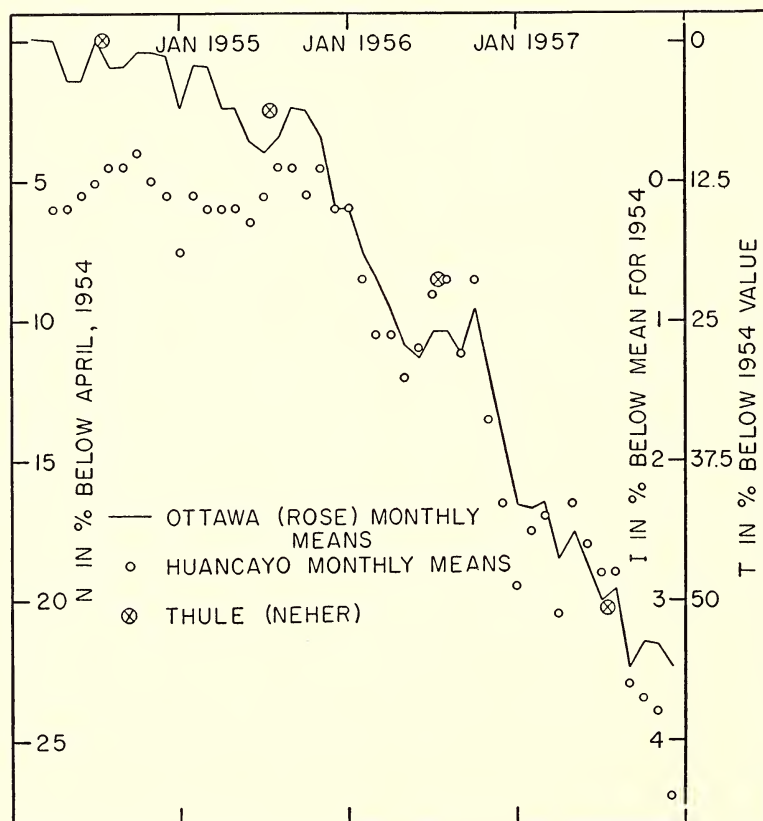


Fig. 9. Neutron intensity (N) at Ottawa, ionization (I) at Huancayo, and ionization under 15 g cm^{-2} at Thule (T).

Old cosmic-ray program. Compton-Bennett meters were satisfactorily operated throughout the report year at Godhavn (Greenland), Climax (Colorado, U. S.), Ciudad Universitaria (Mexico, D. F.), Huancayo (Peru), Christchurch (New Zealand), and Fredericksburg (Virginia, U. S.).

The scaling and reduction of records including the tabulation of bihourly means of ionization corrected for barometric pres-

sure have been completed for Huancayo and Fredericksburg through April 1958. From the records scaled at Christchurch the reduction of daily means has been effected through June 1957. To cooperate in the United States program for cosmic-ray research in the International Geophysical

Year, tabulations of corrected bihourly means of cosmic-ray intensity have been forwarded to the four IGY World Data Centers. It was originally planned to cooperate with the U. S. IGY cosmic-ray program by making available to World Data Centers (WDCs) only the tabulations of corrected bihourly values of cosmic-ray intensity from Huancayo and Fredericksburg. The results at Christchurch were to be made

available to WDCs from New Zealand. The stations at Godhavn, Climax, Mexico, and Derwood (large ionization chamber) were to be operated for solar-flare patrol observations and unusual magnetic storm effects.

The U. S. IGY Technical Panel for Cosmic Rays emphasized the importance of results from the program for continu-

the reduction of as many as possible of these data.

On the basis of the recommendation of the Panel and the approval of the U. S. IGY National Committee a modest grant was made, to provide the necessary additional assistance for the reduction of the data.

Large ionization chamber. The large

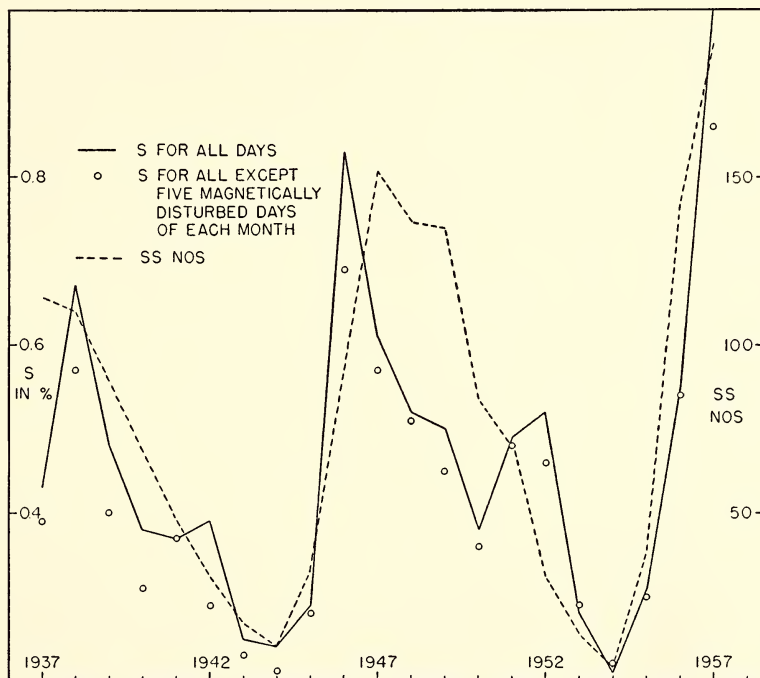


Fig. 10. Yearly pooled standard deviation (S) of daily means of cosmic-ray intensity at Huan-cayo, and yearly mean sunspot numbers (SS).

ous registration of cosmic-ray intensity, begun by the Carnegie Institution of Washington between 1936 and 1938, and noted the fact that this program had provided the only continuous record of cosmic-ray variations over so long a period.

The Panel therefore urged that the data from Godhavn and Ciudad Universitaria (Mexico City) be completely reduced for the duration of the International Geophysical Year (July 1957–December 1958). The data from Godhavn for the period January 1951 to July 1957 not having been reduced, the Cosmic Ray Panel also urged

cosmic-ray ionization chamber was maintained in essentially continuous operation at Derwood during the report year. No solar-flare effects have been observed since February 23, 1956.

Cooperation in operation of cosmic-ray meters. The successful operation of Compton-Bennett cosmic-ray meters over a long period at so many stations has been possible only through the wholehearted and unselfish cooperation of several organizations and individuals. We wish to express our appreciation to the following organizations for the operation and main-

tenance of cosmic-ray meters: the Danish Meteorological Institute and the staff of its Godhavn Magnetic Observatory at Godhavn, Greenland; the U. S. Coast and Geodetic Survey and the staff of its magnetic observatory at Fredericksburg, Virginia; the High Altitude Observatory of the University of Colorado and its staff at Climax, Colorado; the Instituto Nacional

de la Investigación Científica and the Universidad de Mexico, Mexico, D. F.; the Government of Peru and the staff of its Instituto Geofísico de Huancayo for making available the Compton-Bennett records from Huancayo; and the Department of Scientific and Industrial Research and the staff of its Magnetic Observatory at Christchurch, New Zealand.

LABORATORY PHYSICS

NUCLEAR PHYSICS

N. P. Heydenburg and G. M. Temmer

During the past year, we have once again returned to the domain of the lighter nuclei and "proper" nuclear reactions, after a most unusual and rewarding interlude of about three years spent with Coulomb excitation of most elements up through uranium. Although a few groups still continue to follow up more detailed problems raised in this electric excitation of nuclei, we felt that within the means at our disposal, and within the limitations set by our own interests, we had come to a definite stopping point. Looking back, we have measured the energies and absolute transition probabilities (lifetimes) of about 200 electromagnetic transitions in some 120 nuclear species, employing about 75 enriched isotopic targets for definite assignments and greater accuracy. About one-third of these transitions represented previously unknown cases, and essentially none of the transition rates had been measured before. It is clear, therefore, that we gained a great deal of insight into the systematic behavior of these transitions, especially in the even-proton-even-neutron nuclei, where without exception the first-excited state of spin parity 2^+ was excited. Very clear evidence was obtained for the existence of rotational states in both odd and even heavy deformed nuclei beyond europium as well as for the vibrational states in the lighter nuclei with spherical equilibrium shapes. In fact, the very sharp line of demarcation between them was located within two neutron numbers (151

and 153). The details of most of these developments have been discussed in the last three annual reports. With the study of one of the last (and most exotic) elements, to be discussed below, we have essentially completed our work in Coulomb excitation of nuclei with α particles up to 7 Mev. Some of the future developments in this field seem to lie with the heavy-ion accelerators such as have begun to operate at Berkeley and at Yale, heavy ions, because of their higher charge, permitting the bombardment of lighter elements without danger of purely nuclear interference.

Coulomb Excitation of Xenon

In our early results on the Coulomb excitation of normal xenon, the isotopic assignment of the observed γ rays was not clear on account of the many isotopes present in normal xenon. Enriched isotopic samples, prepared by thermal diffusion at Yale, were made available to us, and in cooperation with G. F. Pieper and C. E. Anderson, guest investigators from Yale, the γ -ray spectra of these gas samples were observed when Coulomb-excited by 6.6-Mev α particles. The samples were bombarded in a small chamber separated from the main accelerating tube by a thin nickel window. The γ rays were observed with a sodium iodide detector and 80-channel pulse height analyzer.⁶ By comparing rela-

⁶ This analyzer, which incorporates a quartz delay line memory system, was constructed by Mr. Buynitzky under the guidance of G. F. Pieper. It has operated very well during the past

tive γ -ray intensities in the two enriched samples and normal xenon, the observed γ rays at energies 670, 535, and 445 keV could be assigned to the even-proton, even-neutron isotopes Xe^{132} , Xe^{130} , and Xe^{128} , respectively. These γ rays appear in the de-excitation of the first 2^+ levels which are excited in the Coulomb excitation process. Their energies and intensities vary in a systematic way, characteristic of the approach to a closed shell, in this case to the closed shell at neutron number 82. Because of a slight nitrogen contamination in the samples a γ ray appeared at 875 keV, due to the intense reaction $\text{N}^{14}(\alpha, p\gamma) \text{O}^{17}$. The presence of a possible γ ray due to Xe^{134} which should occur in this energy region was therefore difficult to determine.

Gamma rays were also observed at 290 and 365 keV. The 365-keV γ was assigned to Xe^{131} and is due to the de-excitation of a known level in Xe^{131} at 365 keV. A cascade transition is also known to occur from this level through a level at 80 keV. The observed peak at 290 keV is due in part to the cascade γ ray at 295 keV. Most of the intensity at 290 keV, however, is due to Xe^{129} . A γ ray of this energy has not previously been reported in Xe^{129} .

Level Structure of Na^{22}

Over the last few years the interest in the lighter nuclei has gradually shifted from the discovery of energy levels and their spin and parity assignments to attempts to measure their transition rates or at least relative decay probabilities. That almost all energy levels and many of their characteristics have been discovered by now is not too surprising in view of the limited number of light nuclei (about 20 species) and the large number of Van de Graaff generators in the world (about 50) capable of exciting them. One of the remaining fruitful frontiers is the measurement of level widths (transition probabili-

ties). This approach is complementary to more recent theoretical progress in obtaining nuclear wave functions on the basis of various models and hence making it possible to calculate transition rates between identified nuclear states. As this particular parameter is sensitive to the nuclear models used it gives promise of being able to choose between them.

Our instrumentation for the detection and analysis of pulse-height distributions had been greatly expanded during the previous report year. In addition, we built a fast coincidence system with a resolving time of about 20 millimicroseconds capable of gating the multichannel device with a movable single channel. It allows us to find out, for instance, what parts of a γ -ray spectrum excited in a nuclear reaction are in either prompt or delayed coincidence with a particular transition selected in the single channel. We spent considerable time improving the over-all circuitry, especially on the time compensation part, the device allowing the simultaneous prompt coincidence of all parts of the examined spectrum regardless of pulse height. The usefulness of the entire set-up of course depends upon the proper functioning of this component.

One of the first problems we attacked with our new instrumentation was one we had encountered some four years ago in the nucleus Na^{22} . The Na^{22} nucleus is of the "self-conjugate," odd-odd type: it has equal (odd) numbers of protons and neutrons. Such a nucleus has two even-even isobaric neighbors, in this case Ne^{22} and Mg^{22} , the three nuclei forming what is known as an "isobaric triplet." In analogy with the ordinary spin in spectroscopy, an "isobaric spin" T is introduced, which takes the value 0 if only the central member of the triplet shows a certain configuration, or the value 1 if corresponding configurations occur in all three members of the triplet, the isobaric spin "projection" taking on values 1, 0, and -1 . The existence of the $T=1$ variety of multiplets

year, and has made possible many observations that could not have been made with a single-channel analyzer.

is evidence for the charge independence of nuclear forces, i.e. the basic identity of neutron-neutron, proton-proton, and neutron-proton forces. Particular interest attaches to the lowest excited state of the central member having $T=1$ (Na^{22}), since this corresponds to the ground states of the two even-even neighbors. It must therefore necessarily have spin parity 0^+ . Now the ground state of Na^{22} has a measured spin of 3^+ ; therefore, if the first-excited state of Na^{22} at 593 kev, which we discovered several years ago, were the analog state in question, its de-excitation would involve a spin change of three units with no change in parity, that is, a magnetic octupole transition. Such a transition should have a lifetime of about 0.1 second or longer.

Upon re-examining the reaction $\text{F}^{19}(\alpha, n\gamma)\text{Na}^{22}$, one of the first facts we discovered was that the lifetime of that transition was only 0.266 microsecond, thus ruling out spin 0^+ and hence the $T=1$ character for the 593-kev state. We further found that the 593-kev radiation was in coincidence with a low-energy γ ray of 73 kev which had to come from a state at 666 kev. Figure 11 shows both the coincident pulse-height distribution when the single channel was placed on the peak corresponding to 593 kev, revealing a pronounced peak at 73 kev, and the coincidence rate as a function of artificial delay introduced into the 73-kev detector, showing the exponential decay of the 593-kev state with a half-life of 0.266 microsecond. Several pieces of evidence point to this state at 666 kev as the one we had been looking for, not the least important of which is the remarkable agreement of its location with that calculated from the systematics.

In the familiar series of mirror nuclei known as Wigner elements the energy difference between adjacent isobars can be accounted for entirely by the Coulomb energy difference due to the one additional proton. In fact, one of the classic methods for the determination of nuclear

radii is based on the observed energy differences. Now for a self-mirrored nucleus such as Na^{22} one evidently has to use the difference between the analogous 0^+ states of neighboring isobars, the ground state of Ne^{22} and the newly discovered state of Na^{22} at 666 kev in our case. In figure 12 the systematic plot of Coulomb energy differences as a function of $A^{2/3}$ for these

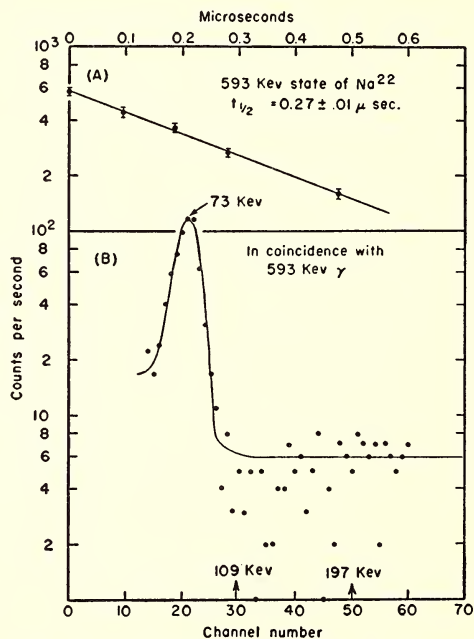


Fig. 11. (A) Coincidence rate vs. artificial delay introduced in the 73-kev channel, showing a 0.266-microsecond half-life for the 593-kev state of Na^{22} . (B) Pulse-height spectrum in coincidence with 593-kev radiation in the single-channel gate, showing pronounced 73-kev peak. It is this peak height as a function of introduced delay that is plotted in (A).

self-conjugate nuclei between major shells shows excellent agreement for Na^{22} . The straight-line relationship indicates the dependence of the nuclear radius upon the cube root of the atomic weight.

We also excited Na^{22} in another manner, by bombarding Ne^{20} with He^3 ions, inducing the $\text{Ne}^{20}(\text{He}^3, p)\text{Na}^{22}$ reaction, and detecting the emerging protons in a thin scintillator in coincidence with γ radiation. A great many proton groups are revealed, and they fit into the general level scheme

as summarized for all reactions in figure 13. It should be emphasized that neither reaction is of the self-conjugate type, such as $\text{Mg}^{24}(d, \alpha)\text{Na}^{22}$, where all nuclei and particles involved are self-mirrored. This means that, because of the expected conservation laws applying to the isobaric spin quantum number, only $T=0$ states should be excited in the latter reaction, since in Mg^{24} the deuteron and α particle

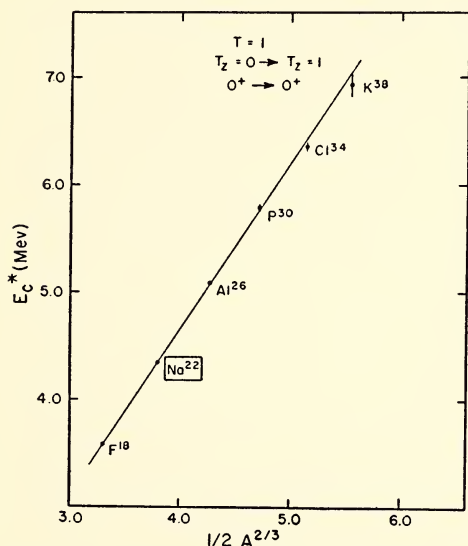


Fig. 12. Systematic plot of Coulomb-energy differences due to addition of one proton for the group of nuclei having equal (odd) numbers of neutrons and protons vs. $A^{2/3}$. Corresponding members of the $T=1$ isobaric triplets are used. Na^{22} is seen to fall exactly as expected. The near-straight-line variation between nuclear shells at O^{16} and Ca^{40} indicates the dependence of the nuclear radius on the cube root of the atomic weight A and the correctness of ascribing the energy difference to the electrostatic energy involved in adding one proton to these nuclei.

are all in their $T=0$ ground states, while our two reactions allow both $T=0$ and $T=1$ states to be formed in Na^{22} (since F^{19} , He^3 , and the neutron have $T=1/2$). As can be seen from figure 13 the (d, α) reaction excites all low-lying states of Na^{22} except the new one at 666 kev. This constitutes additional strong evidence for its $T=1$ character.

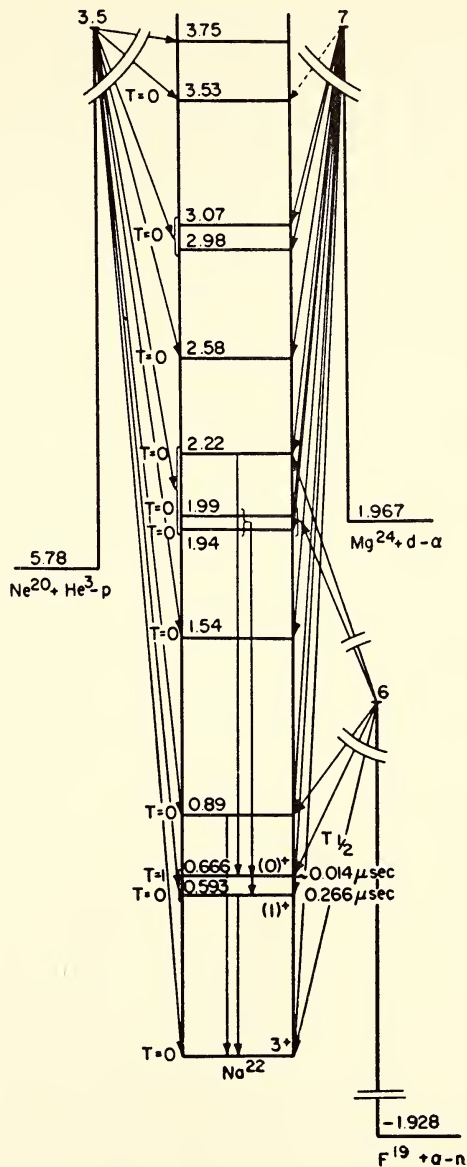


Fig. 13. Summary of present knowledge on level structure of Na^{22} , showing information from both reactions used by us, and the $\text{Mg}^{24}(d, \alpha)\text{Na}^{22}$ "self-conjugate" reaction capable of exciting only $T=0$ states. Tentative spin assignments are shown in parentheses.

Atomically Polarized Ion Source (APIS)

Considerable interest in both nuclear and elementary particle physics has centered over the past few years in the production

and use of polarized particles for the study of nuclear interactions. The preparation of polarized beams has thus far always involved a first polarizing event, either a nuclear reaction such as the (d, d) or the $\text{Li}^7(p, n)$ reaction at low energies, or an elastic or inelastic scattering event at high energies (of the order of 100 Mev or above). The detection of preferential spin orientation in outgoing beams after these encounters then involves another interaction, such as a scattering of protons or neutrons from helium, where the analyzing power is very well understood. Unfortunately such "double scattering" experiments suffer greatly from lack of intensity, since the "beams" of polarized particles are expressed in 10^5 to 10^8 particles per cm^2 per sec rather than in microamperes (10^{12} per sec) as is appropriate for nuclear studies. It is clear that a beam of polarized particles is intrinsically capable of detecting higher "moments" of a nuclear encounter than an unpolarized beam, which of necessity averages over all spin orientations, thus sacrificing a certain amount of information content.

A number of suggestions have been made recently in an attempt to improve the situation with regard to polarized-particle beams. Most of them have centered around the idea of polarizing, say, protons in the *atomic* state, thus having the benefit of working with an atomic rather than a nuclear Bohr magneton. In principle, then, the same trick is to be tried as is employed for the polarization of radioactive nuclei through the intermediary of the strong hyperfine interaction between the electron cloud and the nucleus imbedded within it, as was described in Year Book 54. Therefore a more or less conventional atomic beam experiment of the Rabi type with atomic hydrogen or deuterium is contemplated, involving an arrangement of magnetic field gradients and uniform magnetic fields, together with judiciously placed mechanical stops and radiofrequency-induced transitions in such a way as to per-

mit only one of the hyperfine components to pass through the array. This component then consists of, say, hydrogen atoms polarized transversely to the beam direction, at thermal energies. This implies polarized hydrogen nuclei (protons), since they have a definite orientation with respect to the electron spin (parallel or antiparallel). If this beam is now ionized in some manner, as by electron bombardment, a beam of essentially complete proton polarization is obtained. In order to verify the existence of polarization, it is necessary to perform a nuclear experiment of some sort, such as the above-mentioned scattering from helium at about 2 million electron volts. In other words, the atomic beam source will have to be placed in an accelerator before we shall know whether any depolarizing influences have been overlooked in the scheme.

Vernon W. Hughes and Charles W. Drake, of Yale University, have suggested a collaborative effort to attempt to produce this type of polarized beam, making use of our uniquely suited facilities. Because of the vast dimensions of our pressurized Van de Graaff generator it is possible, without giving further thought to miniaturization or reduction of power requirements, to install a conventional atomic beam apparatus in our high-voltage terminal. Neither space (about 3 cubic meters) nor power requirements (about 7 kilowatts for the source proper) are problems in our accelerator. Furthermore, our ability to accelerate protons up to 2 Mev, the region of immediate interest, without having to pressurize the generator will greatly facilitate the initial alignment procedure. No other accelerator of this type exists in the world at present.

Last, but not least, the uncluttered nature of our machine schedule, the lack of obligation to graduate students working on theses, or to other investigators committed to different research projects, makes our situation particularly suited to this kind of pioneering effort.

The source proper is being constructed

and assembled at Yale University, and will be subjected to preliminary tests there before being installed in our high-voltage terminal. We expect to have preliminary indications of the feasibility (or lack thereof) during the next report year.

BIOPHYSICS

E. T. Bolton, R. J. Britten, D. B. Cowie, and
R. B. Roberts

INTRODUCTION

It is our privilege to study life's most fascinating problem—the nature of life itself. Such a tremendous subject must of course be broken down into lesser parts which can be comprehended, or the investigator would be overwhelmed. In our dissection of the problem we have asked a subsidiary question, but still a profound one. Can the physical attributes of life be explained in terms of the properties of atoms and molecules?

Training in physical science does not qualify us in any special way to question whether there is a spiritual as well as a physical aspect of living creatures, whether a dualism exists. It is, however, within our capabilities to attempt to discern limitations to our understanding of the physical and chemical processes of life. Limitations here might be comparable to the conceptual limitations encountered in attempting to interpret atomic physics without the help of quantum theory.

When the problem of the nature of life is restricted in this way, the biological material chosen as most amenable for study needs only to have the essential chemical and physical properties of life, and need not exhibit any mental or spiritual capacities. But what are the essential chemical and physical properties of life? If life had been synthesized *de novo* from nonliving ingredients in a beaker on our laboratory bench, how would we recognize it?

One obvious quality of living organisms is their capacity for growth. Another is reproduction. Yet those qualities alone do not define life, and in framing a defini-

tion it is necessary to choose words and concepts that include such creatures as mules, which cannot reproduce, and that exclude salt crystals, which can grow. Furthermore, such objects as seeds, and perhaps even viruses, should be included. Both have the capability of catalyzing the synthesis of more of their kind even though they may not exhibit growth or other metabolic activity for long periods of time. Thus, there seems to be no simple answer to how newly created life might be recognized. On the other hand, there are several useful central concepts that seem appropriate to considerations of life at the macromolecular level. Among the most vital are autocatalysis, and its implications for growth and reproduction, and the capacity for evolution, the ability to give rise to persistent lines even though they may differ from parental stock. Also, a minimum level of "organization" seems to be involved—sucrose molecules do not qualify as living, except in poetic imagery; and viruses, which are nucleoprotein molecules, perhaps may.

The components of living cells that may fall within the scope of these speculations about the nature of living matter are deoxyribose nucleic acid (DNA) and ribose nucleic acid (RNA) or perhaps these materials in association with protein (DNP and RNP). Certainly these together qualify as a living system when they are within the protoplasm of a growing cell. Accordingly, a large fraction of our interest has continually been focused on the biosynthesis of these large and complicated molecules. The processes supplying the energy and the material required for synthesis are complicated, but they can be understood in principle. The processes by which their building blocks are assembled in proper order, however, remain obscure. Even so, there is no indication that the usual forces of chemistry will fail to provide an adequate interpretation.

In this year, much more than in the years gone by, we seem to be approaching

a climax in a long story. Almost twenty years ago Brachet and Caspersson showed that RNA was prominent where or when protein synthesis was in progress. Quite recently it has been demonstrated that in virus infections it is the nucleic acid and not the protein part of the virus that specifies the protein of the virus progeny. Another observation of recent years is that most of the RNA of cells is held in large part in granules of nucleoprotein ranging from 1 to 4 million in molecular weight. These particles, recently named ribosomes, are found in such widely varied sources as bacteria, pea seedlings, and rat liver. Accordingly, there has been a growing belief that the ribosomes form the site and machinery for protein synthesis. This theory has been strengthened by the finding in several laboratories that freshly incorporated amino acids appear first in protein associated with the ribosomes and only later in other protein. Furthermore, cell-free preparations of purified ribosomes (with the addition of certain enzymes and cofactors) have shown some incorporation of amino acids into protein.

Nevertheless, a considerable measure of doubt about the mechanism of protein synthesis still remains. The observed incorporation into cell-free ribosomes requires only the addition of one amino acid at a time and thus suggests exchange. Two other laboratories have reported during the year that fragments of cellular membranes carry out a more extensive synthesis of protein and do in fact require the presence of all the amino acids. Possibly these findings may be reconciled if it turns out that the ribosome must unfold on the surface of the membrane to be active as a template for synthesis. In any event, further work is needed before the issue is settled.

This year's work in our Biophysics Section has again centered around protein synthesis. Ion-exchange columns, radioactive tracer methods, and the Spinco Model L centrifuge were used to study the properties of the ribosomes and the kinetics

of their formation. With the new Spinco Model E analytical centrifuge we are attempting to understand the biological significance of the different sizes of ribosomes. Bacteria rapidly growing in a broth medium contain more of the smaller particles than bacteria growing in a glucose-salts medium, and cells treated with chloramphenicol in order to halt protein synthesis contain mostly one class of ribosome. Such results, although only a preface to chapters yet to be written, show that the ribosome size distribution can serve as an indicator of the protein-synthesizing ability of the cell. It is hoped that current investigations will reveal whether the size distributions are causes or effects of altered protein-synthesizing capacity. It is already clear that the ribosomes contain protein distinct from the other protein of the cell and that the ribosomes cannot disintegrate to supply the other protein. On the contrary, synthesis of the ribosomes appears to proceed at quite a leisurely rate. Studies with radioactive tracers indicate that the nucleic acid portion of the ribosome is derived from nucleic acid macromolecules which are initially chemically associated with little, if any, protein. This result suggests that ribosomes may grow, and move from one size class to another, by combining with protein and nucleic acid macromolecules rather than by adding small molecules.

The use of amino acid analogs has provided other information about the mechanism of protein synthesis. The cells cannot perfectly distinguish these analogs from the usual amino acid, and they are incorporated into the protein. Furthermore, they are incorporated into different proteins in the same proportion, suggesting that the mechanism for amino acid selection does not differ from one protein to another. A given analog affects the activity of different enzymes to different degrees. This observation suggests that the amino acid complement at the active sites of an enzyme may be examined by study-

ing the sensitivity of the enzyme to a spectrum of analogs.

Other studies have been carried out with *Hydra* and *Planaria*. Collaboration has continued with Drs. Flexner, of the University of Pennsylvania, in a study of protein synthesis in mouse tissues. With these more highly organized animals the problems are more complicated, as interactions between different types of cells are involved. The experiments show the usual processes of synthesis as they occur in different animals and different tissues and, in addition, provide an introduction to the effects of higher levels of organization. It is encouraging to find that much of the experience gained with microorganisms is useful in dealing with complex animals.

The experimental work leading to these conclusions is described in detail below.

FRACTIONATION METHODS

A single cell of the bacterium *Escherichia coli* contains roughly 10,000 ribosomes. As was mentioned above, there are numerous indications that the ribosomes play some important role in protein synthesis, but additional experimental evidence is needed to prove the point conclusively and to supply information on the mechanism involved. One approach is to study the properties of the ribosomes: their stability, their composition, and, if possible, their structure. Another is to correlate the proportion of different sizes of ribosomes with the metabolic state of the bacteria. Still another is to observe the incorporation of various tracers and their transfer from one class of molecules to another.

As a first step it is necessary to break the cells and to separate out the various types of molecules. Suppose, for example, that one class of particles is the precursor of another, or of the soluble protein. Kinetic measurements of tracer incorporation will show this clearly if the various classes can be separated. Thus, chemical fractionation with various extracting agents separates the small molecules from lipid, protein,

and nucleic acid, and kinetic measurements show that the small molecules serve as precursors for the large ones.

Chemical fractionation does not, however, distinguish free nucleic acid and protein from nucleoprotein. Nor does it distinguish which components are bound in the cell wall or membrane. To distinguish these classes we have tried various centrifuging techniques, electrophoresis, and cellulose ion-exchange columns. Chemical analysis of the fractions obtained by these methods is then useful to determine the composition and purity of the fractions. Also, the fractions obtained by one method can be analyzed further by another.

The French pressure cell has proved very satisfactory in breaking the cells. Large quantities of cells (10 g) can be broken rapidly, and the breakage is 99 per cent complete. When necessary, the residual unbroken cells can be removed by centrifuging four times successively for 2 minutes in the Servall centrifuge to give a cell-free juice. This step also removes an appreciable fraction of the large fragments of cell debris.

The bacterial juice obtained from the pressure cell has a low viscosity, as DNA is fragmented on passing through the cell. Low viscosity is usually an advantage, for the succeeding fractionation steps are difficult when a mass of jellylike DNA is present. If unbroken DNA is required, a different way of breaking the cells must be used. Grinding with alumina or osmotic shock of lysozyme-treated cells will yield intact DNA, but another method has proved far superior.

In this method the cells are washed in a magnesium-free tris-succinate buffer (TS) 0.01 M adjusted to pH 8. Lysozyme (0.1 mg/ml), ethylenediaminetetraacetic acid (EDTA) (0.01 M), and sodium deoxycholate (1 per cent) are added. The lysozyme and deoxycholate act in conjunction to lyse the cells promptly and give a viscous fluid from which the DNA can be removed with a stirring rod after the addition of ethanol. The usual meth-

ods are then followed to purify the DNA.

In most of our work the undegraded DNA is not required and the pressure cell juice is used. Cell walls and debris are quite effectively removed by a single centrifugation for 15 minutes at 100,000*g*. There are some indications that the cell-wall fraction is not homogeneous. If the pressure cell juice is spun twice at 40,000*g* for 15 minutes the second pellet which contains the smaller fragments has almost

in 2 hours) and small bits of cell membrane (this pellet has a high lipid content). The contamination is best shown by the column analysis described below. The supernatant fluid contains soluble proteins, free RNA and DNA.

A further purification of the ribosomes is achieved by resuspending this pellet and spinning at 40,000*g* for 15 minutes. The proteins and membrane contaminants do not resuspend but remain aggregated and

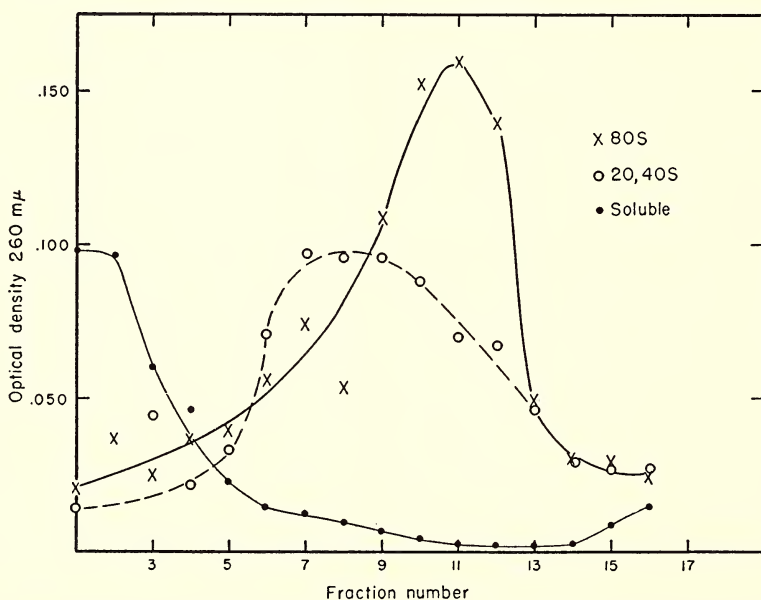


Fig. 14. Fractionation of particle preparations using the swinging bucket centrifuge. Five-tenths milliliter of suspension is placed on top of 4.5 ml sucrose gradient in the centrifuge tube. After 45 minutes at 100,000*g*, 0.3-ml fractions are taken off from the top with a pipet.

twice the proportion of lipid found in the first pellet, indicating that it may contain more lipoprotein "membrane" and less of the structural "wall." The nucleic acid content of these fractions is low, indicating only a small contamination by ribosomes.

Most of the wall or membrane having been removed, the remaining fluid is centrifuged at 100,000*g* for 1½ to 2 hours; the resulting pellet is (by definition) the microsomal fraction. It contains ribosomes together with large proteins (roughly 70 per cent of β -galactosidase is sedimented

are sedimented. The ribosomes go into suspension more readily and are subsequently sedimented by centrifuging at 100,000*g* for 2 hours.

Attempts were made to separate the various sizes of ribosomes by choosing an appropriate centrifuging schedule. Thus, the analytical centrifuge showed that material which sediments in 15 minutes at 100,000*g* is richer in the large particles than the pellet obtained by centrifuging (2 hours, 100,000*g*) material remaining in the supernatant fluid after three successive 15-minute 100,000*g* spins. This approach

showed no real promise of giving adequate fractionation.

A better separation of the different ribosomes can be obtained by means of the swinging bucket head for the Spinco Model L centrifuge. Microsome pellets are resuspended and layered on top of a sucrose gradient. After a period of centrifugation, layers are taken off with a pipet. This technique is adequate to demonstrate marked differences in the distributions, depending on the initial material. Figure 14 shows one curve for a resuspended pellet composed mostly of large (80S) particles, another for the smaller particles (20 to 40S), and a third for the nonsedimenting material. The analytical centrifuge shows that the bottom layers are rich in the heavy particles and lack the light particles, whereas the top layers have the opposite distribution.

An entirely different type of fractionation results from chromatography on columns of diethylaminoethyl cellulose (DEAE). Extremely high resolution can be achieved giving a separation of various proteins, as shown in figure 15. Nucleoprotein appears as a prominent peak in the elution diagram of the total cell juice but not in the diagram obtained with the 100,000*g* supernatant fluid (fig. 16). The corresponding ultraviolet diagrams show two main peaks: the first consists of nucleoprotein of high molecular weight which can be spun down in the centrifuge; the second is partly nucleoprotein and partly due to free DNA and RNA still remaining in the 100,000*g* supernatant fluid.

The elution pattern is not sensitive to the size of the particles. The same pattern is obtained whether the microsome pellet is composed mostly of the large (80S) particles or of the smaller (20 to 40S) ones that result from magnesium deficiency or treatment with NaCl. Compare figures 17*A* and 17*B*.

Microsome pellets, when resuspended and analyzed on the column, show the nucleoprotein peak together with a quan-

tity of other protein which depends on the method of preparation (fig. 17). The least-contaminated preparations of ribosomes are those obtained by resuspending

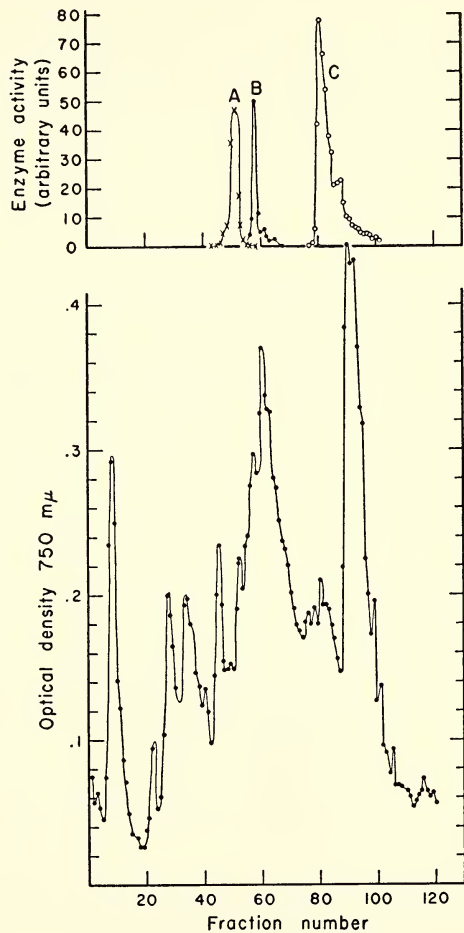


Fig. 15. Cells washed in TSM and broken with French pressure cell; 0.5 g wet weight of cell juice adsorbed on DEAE column (1 cm² × 20 cm) and eluted with concentration gradient 0 to 0.7 *M* of NaCl in tris-succinate buffer plus magnesium. Lower curve, total protein indicated by Folin reaction; upper curve, assay for activity of three different enzymes. One-milliliter samples collected in fraction collector.

a microsome pellet and centrifuging again in the swinging bucket head (fig. 17*C*).

Unfortunately, the column cannot be used to prepare purified ribosomes because the material eluted from the column is very different from that originally ad-

sorbed. When the fractions containing the nucleoprotein peak are centrifuged (100,000g, 2 hours), a colorless glassy pellet is formed which contains approximately 65 per cent of the protein and nucleic acid. This pellet resuspends easily and completely. The analytical centrifuge shows that it contains peaks in the 20 to 40S

to molar NaCl show a reduction in size but no change in composition or elution pattern. This nucleoprotein derived from ribosomes, but having a changed amino acid to base ratio as a result of degradation on the column, is designated CNP to distinguish it from the original NP of the ribosomes. The letter "C" in CNP

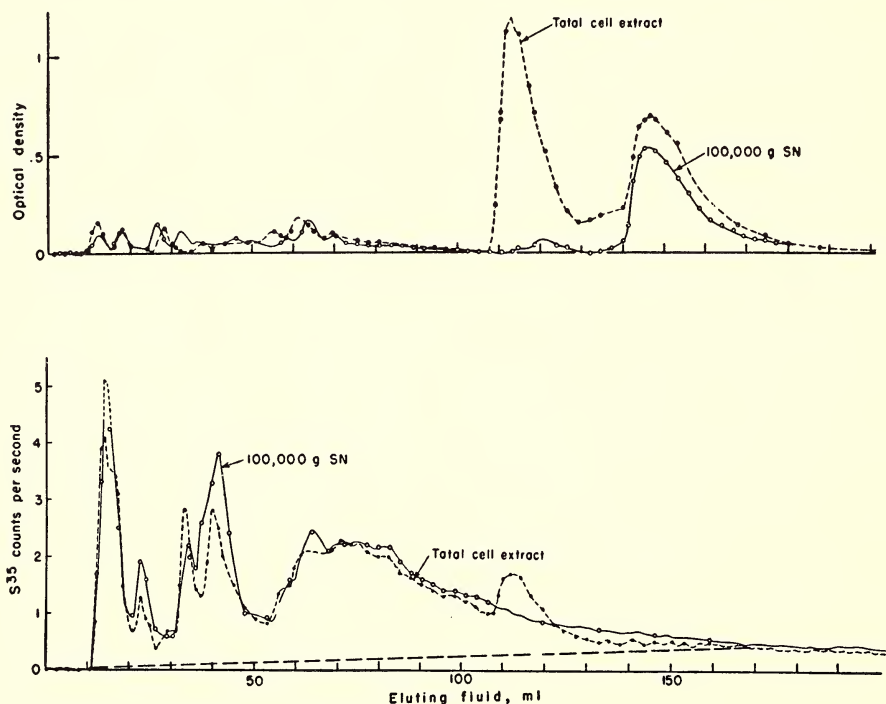


Fig. 16. Elution patterns of total cell juice and supernatant fluid of 100,000g 2-hour spin. Upper curve, optical density at 254 m μ indicating nucleic acid concentration; lower curve, S^{35} radioactivity, indicating protein. Note nucleoprotein peak which is missing in 100,000g SN. Figures 16 through 22 are taken by permission from Roberts, *Microsomal Particles and Protein Synthesis*, published for the Washington Academy of Sciences by the Pergamon Press, 1958.

region, whereas the 80S peak was most prominent in the original material. The ratio of nucleic acid to protein in the pellet (measured by optical density at 260 m μ and S^{35}) is twice that of the starting material, and the elution pattern obtained when the pellet is rerun on a DEAE column is very different (fig. 18).

These changes appear to be caused by the column material and not by the salt of the eluting fluid. Ribosomes exposed

was chosen as a reminder of the method of preparation by elution from a chromatographic column.

PROPERTIES OF RIBOSOMES

The ribonucleoprotein of *E. coli* occurs in several species of particles which can be differentiated by their sedimentation rates in the analytical ultracentrifuge. The proportions of particles in the ribosome species may be varied *in vitro* by altering

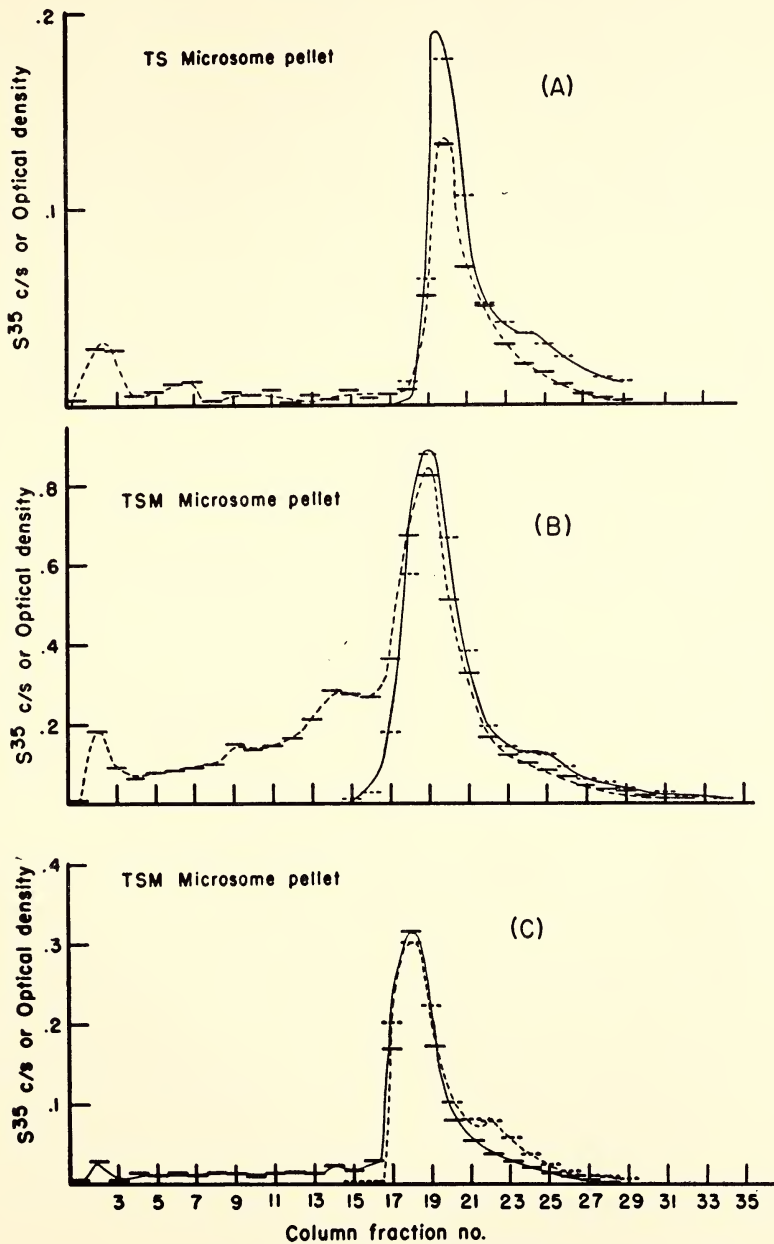


Fig. 17. Elution patterns of microsome pellets. (A) 100,000g 2-hour pellet with magnesium present; (B) same with magnesium lacking; (C) microsome pellet resuspended and fractionated with swinging bucket centrifuge.

the composition of the suspending fluid and *in vivo* by altering the conditions under which the bacteria are cultured.

Figure 19 shows typical sedimentation diagrams of French pressure cell extracts of exponentially growing *E. coli* which were disrupted in the presence of TSM (tris-succinate-magnesium), TS, or TSM and phosphate buffer. Approximate ap-

or relative proportions of components. The sedimentation diagrams of bacterial extracts prepared with the dilute tris-succinate buffers at pH 7.6 remain unchanged as a result of 20 hours' storage at 0° to 4° C (fig. 20). Addition of the chelator EDTA (fig. 21), however, or of the enzyme ribonuclease (fig. 22) removes all components with sedimentation rates

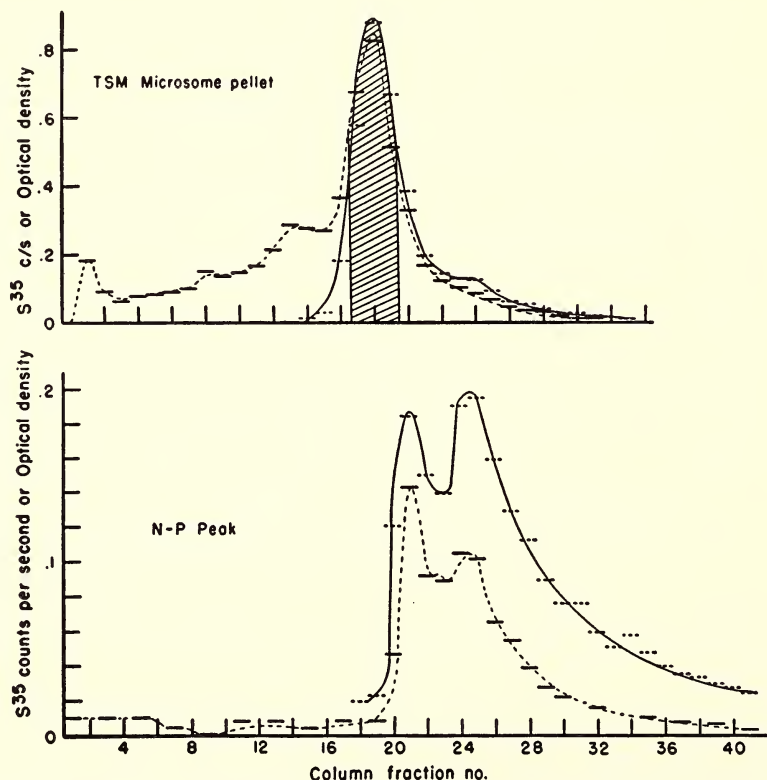


Fig. 18. Nucleoprotein peak of elution pattern (shaded area) spun down and rechromatographed (lower figure). Note change to elution pattern like that of nucleic acid.

parent sedimentation rates are indicated along the abscissa of the upper diagram. It is evident from comparison of these diagrams that more, and larger, components are observed when magnesium has been included in the buffer. The addition of phosphate abolishes the more rapidly sedimenting components. Cysteine and sucrose, which are frequently included in suspending media for subcellular elements, are without effect upon the number

greater than 20S. Deoxyribonuclease (fig. 22), on the other hand, has no apparent effect on the sedimentation behavior of the ribosomes.

The sedimentation patterns of the extracts may also be varied by altering the conditions under which the bacteria are cultured. As is clear in figure 23, plate 2,⁷ marked differences appear even though

⁷ Plates 2 to 6 are grouped between pages 160 and 161.

the cells were harvested and washed in the same TSM medium. Another example shown in figure 23 is the change which results when chloramphenicol is added to

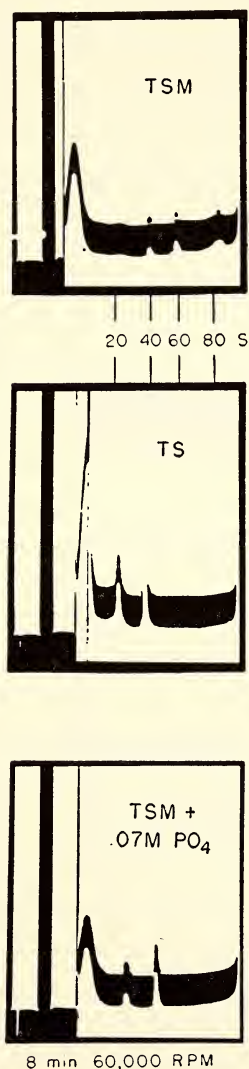


Fig. 19. Sedimentation diagrams of *E. coli* disrupted in various buffer solutions. The concentration of the bacterial juices differed among the runs.

a growing culture of bacteria. These changes seem an important, though as yet uninterpreted, clue to the role of the ribosomes and the mechanism of their formation.

Composition of Ribosomes

Ribosomes purified by differential centrifugation contain 40 per cent protein and 60 per cent nucleic acid by weight. This distribution is based on protein estimations made by chemical and radioactive tracer methods, and on ribonucleic acid measurements made by chemical and spectrophotometric means. Since the average molecular weight of an amino acid residue in the protein is about 108, and the average molecular weight of a nucleotide building block in the nucleic acid is about 325, there are on the average two amino acids for each nucleotide in the ribosome.

The amino acid composition of the ribosomes is also distinctive. These particles contain somewhat less methionine than the average bacterial protein and less than one-fortieth the cyst(e)ine. They probably do not lack cystine entirely, since the enzyme ribonuclease, which contains it, has been found to occur in the ribosomes. They are relatively rich in lysine and poor in aspartic acid. The remaining amino acids appear in roughly the usual proportions. The nucleic acid is probably entirely of the ribonucleic acid type. Chemical tests for deoxyribonucleic acid have been negative.

When the purified ribosomes, which are mostly 40 to 80S particles, are chromatographed by ion exchange on DEAE-cellulose, a single nucleoprotein peak is observed (cf. fig. 17C). This peak (CNP) is a mixture of high-molecular-weight components (sedimentation rates about 20 to 40S). When the mixture is analyzed for protein and nucleic acid an average of only one amino acid is found for each nucleotide. It appears, therefore, that the chromatographic procedure strips off about one-half of the ribosomal protein and decreases the size of the particles. The protein lost from the particles is firmly bound to the exchanger, but may be eluted with sodium hydroxide. When the CNP is again chromatographed it breaks up into two regions (fig. 18). Thus, the chromato-

graphic procedure itself degrades the ribosomes and yields a series of products that indicate heterogeneity among the building blocks of the ribosomes.

The heterogeneity of the ribosomal protein was studied by examining the ion-exchange behavior of ribosomes that had been degraded by 4 M urea, an agent which decomposes hydrogen bonds and

S^{35} radioactivity is illustrated in the upper part of the diagram. Comparison of the S^{35} data shows that urea decomposes the ribosomes and gives rise to several new S^{35} -labeled components. These are eluted much sooner than the CNP of the ribosome. Much of the protein radioactivity actually migrates with the front. This behavior indicates that the proteins

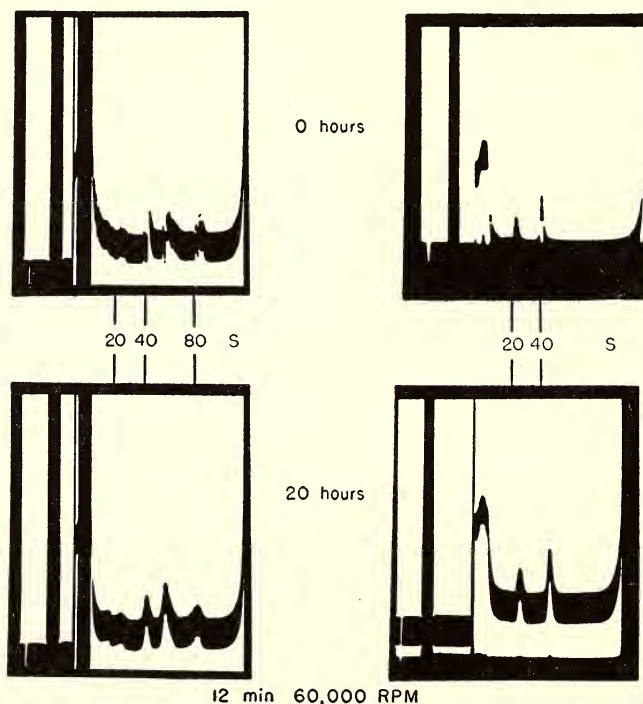


Fig. 20. Influence of storage at 4° C. The suspending buffers (TSM, left; TS, right) also contained 0.25 M sucrose, although subsequent runs have shown that sucrose has no effect on the pattern of components.

denatures many types of protein. Figure 24 shows tracings from an automatic recording device which monitors the radioactivity and ultraviolet absorption of the effluent from an ion-exchange column. Four runs are illustrated: ribosomes that had been exposed to urea for 2 minutes or for 6 hours, and a run of the CNP obtained from the ribosome chromatogram. The CNP solution was centrifuged at 100,000*g* for 2 hours and the pellet was suspended in 4 M urea for 2 hours before chromatography was started.

are uncharged or are positively charged at pH 7.6. When the CNP is urea-degraded and again chromatographed nearly all the labeled protein migrates with the front. Thus, the CNP appears to be composed of protein subunits which are almost entirely basic or uncharged in 4 M urea at pH 7.6. The particular ribosome preparation used for these comparisons was contaminated with nonribosomal proteins, which, even in the absence of urea, are eluted earlier than the CNP (uppermost chromatogram). However, ribosome prep-

arations purified by two cycles of differential centrifugation contain very little contaminating material. When such ribosomes are degraded with urea they yield

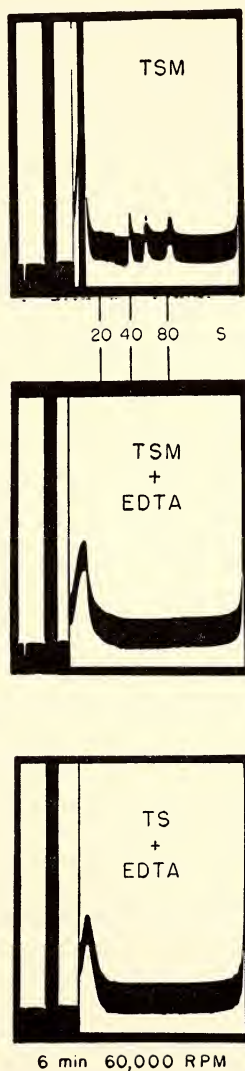


Fig. 21. Effect of EDTA on the sedimentation diagrams of *E. coli* juice. The two lower diagrams are from preparations containing one-half as much material as those for the upper pattern.

chromatograms essentially like those in figure 24.

When ribosomes are treated with urea a clear solution results, but when the CNP is treated with urea part of the protein

precipitates. Thus, still another class of protein may be distinguished, and it may be inferred that urea degradation gives rise to different products, depending on the complexity of organization of the starting material.

The lower part of figure 24 shows the ultraviolet-absorption distribution along the four chromatograms. It is evident that

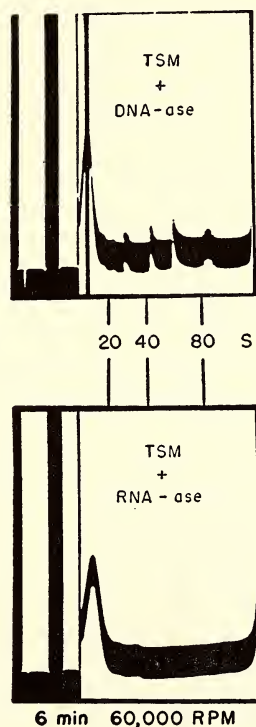


Fig. 22. Effect of nucleases on sedimentation diagrams. The lower pattern is from a preparation one-half as concentrated as that of the upper diagram.

urea brings about dramatic alterations in these patterns. When the treatment has been brief (2 minutes at room temperature) the location of the ultraviolet-absorbing material shows that it is still highly polymerized. This region is now devoid of protein and contains only acid-precipitable ribonucleic acid. If urea treatment of ribosomes or CNP is allowed to proceed for several hours the ultraviolet-absorbing material is no longer acid-precipitable and

the ion-exchange elution pattern resembles that for a mixture of nucleotides of low molecular weight. The degradation of ribonucleic acid in this fashion implies that the ribosomes and also the CNP contain ribonuclease activity. To test this implication yeast ribonucleic acid was added to urea-degraded ribosomes or CNP after the bacterial nucleic acid had become completely acid-soluble. The yeast nucleic acid

migrates with the front, after urea degradation of CNP. These results are illustrated in the center of figure 24. After urea treatment, ribonuclease and also all the rest of the proteins that move with the chromatogram front are precipitable by trichloroacetic acid, and may be redissolved at pH 5 with 0.1 M acetate buffer. The ribonuclease retains full enzymatic activity after this treatment. Thus, in

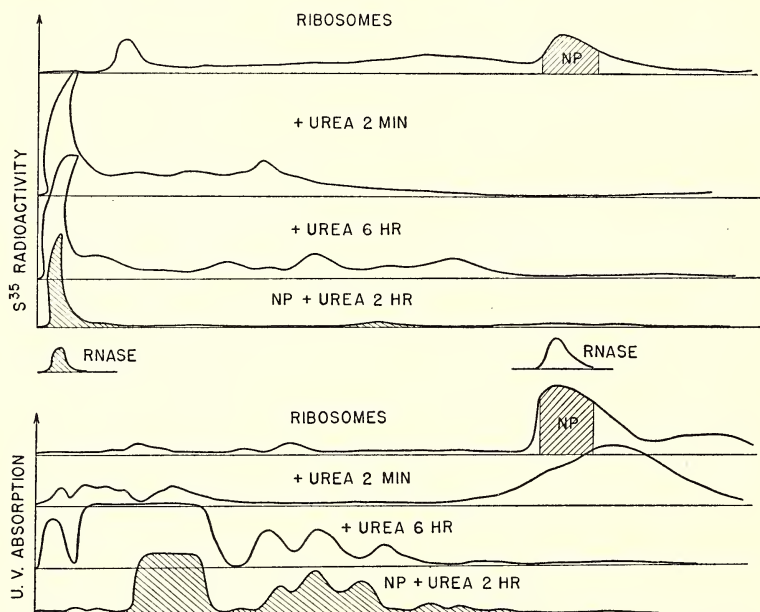


Fig. 24. Heterogeneity among the proteins of ribosomes and the destruction of ribosomal nucleic acid by ribonuclease. Description of these data in the text.

was hydrolyzed by both preparations. Yeast nucleic acid was not degraded by either preparation in the absence of urea. Hence, it was concluded that *E. coli* contains ribonuclease, buried in the ribonucleoproteins, and inactive until the particles are disrupted by the action of urea. Measurements to date indicate that all the *E. coli* ribonuclease is contained in ribosomes. Thus, the enzyme ribonuclease numbers among the various protein subunits of the ribosome.

Further work has shown that the bacterial ribonuclease chromatographs exactly like the CNP before urea treatment, and

chromatographic behavior on DEAE-cellulose and solubility after trichloroacetic acid precipitation the ribonuclease of *E. coli* resembles the ribonuclease of beef pancreas. The demonstration of the latent ribonuclease activity of *E. coli* ribosomes confirms and extends the finding of Dr. David Elson, of the Weizmann Institute of Science (Israel), who also used urea to degrade the ribosomes of *E. coli*.

KINETICS OF INCORPORATION INTO MACROMOLECULAR FRACTIONS

In order to examine the precursor-product relationships among the macromole-

cules of the cell and to assess the possible role of the ribosomes in the synthetic processes of the cell, kinetic studies have been made of radioactive-tracer incorporation into cell fractions separated by means of ion exchange and the ultracentrifuge.

The kinetic studies of protein synthesis using S^{35} as a tracer are still in a preliminary stage. Exponentially growing cells were exposed to the tracer for varying periods of time and then broken and their constituents separated by centrifugation and chromatography. The specific radioactivity of the protein fractions was measured by TCA-precipitable S^{35} and Folin reaction color. When the cells are exposed to the tracer for a prolonged period (steady state) the specific radioactivity varies throughout the chromatographic elution pattern by a factor of roughly 3, being lowest in the nucleoprotein fraction. These variations are simply due to differences in the sulfur content of the different proteins. Alternatively, cells were grown for three generations in a nonradioactive medium after exposure to the tracer. In this treatment any intermediates that have a rapid turnover should lose their radioactivity. The resulting "persistent pattern" was entirely similar to the "steady-state pattern," and no protein components could be identified as intermediates.

Growing cells were also exposed to the tracer for short periods. After a 4-minute exposure the resulting "pulse pattern" was similar to the "steady-state pattern" except that the radioactivity of the nucleoprotein peak was only half that expected from the "steady-state pattern." A similar result was obtained with cells exposed for 4 minutes to a mixture of C^{14} -labeled amino acids.

It appears from these observations that the nucleoproteins (i.e. CNP) of the ribosomes are not precursors of other proteins of the cell. Possibly, however, newly formed proteins might still be found in association with the ribosomes after breaking of the cells. To test this possibility, a

100,000g, 2-hour pellet containing ribosomes (and contaminating protein) from pulse S^{35} -labeled cells was analyzed on the column. It was found that the traces of protein eluting at the same salt concentrations as the bulk of the cellular protein had in fact the same specific radioactivity as the total protein of the cell—not the high specific activity indicative of precursors. This failure to observe precursor proteins in association with the ribosomes is not conclusive evidence that such an association does not exist. On the one hand the turnover rate could be so high that very much briefer pulses would be required for the observation, or on the other hand the association may be so labile as to be destroyed by breakage of the cells.

The search for precursors of nucleic acid using P^{32} as a tracer has been more rewarding. Figure 25 shows the nucleic acid region of the elution patterns obtained with cells exposed to the tracer for increasing periods of time. The radioactivity appears first in a distinct fraction of this region, and later in the other fractions. In the steady-state and persistent patterns the phosphorus radioactivity was proportional to the amount of nucleic acid measured by the optical density at 260 m μ . Thus the DEAE column is capable of resolving the nucleic acid and nucleoprotein into fractions that have the kinetic behavior of precursors and products. Similar kinetic differences were observed earlier in this laboratory by E. H. Creaser, using ECTEOLA columns to analyze alcohol-extracted nucleic acid.

It has not been possible, as yet, to separate the precursors from the nonnucleoprotein RNA and the DNA, owing to the overlap of the elution patterns. A number of chemical and physical characteristics of the precursors, however, have been determined. The precursors are principally macromolecular RNA and together amount to about 10 per cent of total RNA of the cell, as shown by the following observations.

The precursor peaks are TCA-precipitable after elution from the column. They become TCA-soluble at about the same rate as yeast RNA when exposed to beef pancreatic RNAase.

itable P^{32} -labeled RNA has an average sedimentation rate about half that of the RNA in the nucleoprotein particles.

The quantity of RNA present in the form of the precursor is probably small,

DEAE COLUMN ANALYSIS OF *E. COLI* AT EARLY TIMES AFTER ADDITION OF P^{32}

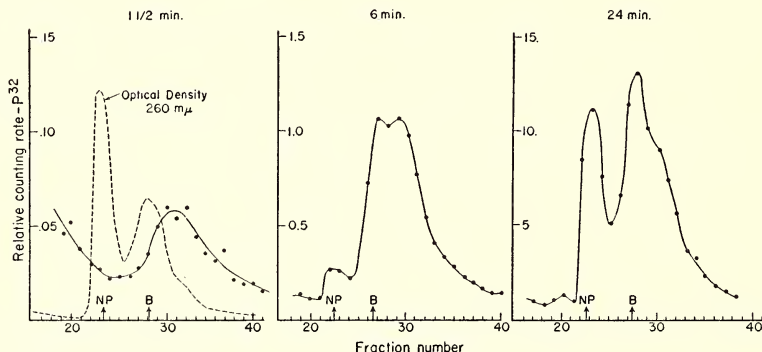


Fig. 25. The labeling of macromolecular nucleic acid at early times after the addition of P^{32} to growing cells. Each curve shows the incorporated P^{32} eluted over the range from 0.35 to 0.75 M $NaCl$, using a DEAE-cellulose column. The broken curve at the left shows the pattern of the ultraviolet-absorbing material. Since this pattern is identical for all the runs the locations of the two ultraviolet-absorbing peaks have been simply marked NP and B.

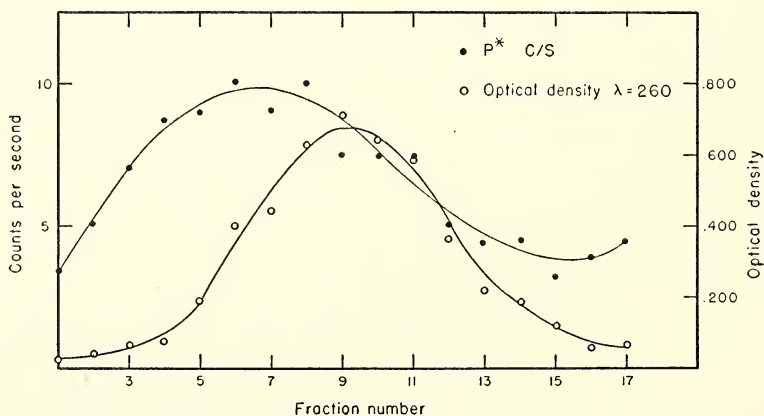
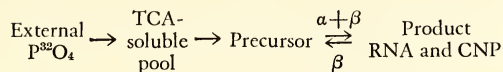


Fig. 26. Sedimentation of P^{32} incorporated into macromolecules during 4 minutes' exposure of growing cells to $P^{32}O_4$. The counting rate of the cold TCA precipitate and the optical density at 260 $m\mu$ are shown after centrifuging 45 minutes at 125,000 g in the swinging bucket rotor. Initially a small sample was loaded over a sucrose gradient in the centrifuge tube.

Figure 26 shows the results of analysis by means of the swinging bucket head in the ultracentrifuge (as described earlier in this report) of growing cells exposed to P^{32} for 4 minutes. The TCA-precip-

since the steady-state and "persistent" labeling show uniform specific activity throughout the elution pattern. Alternatively, the precursor might be large and circulation might occur as indicated in the

following diagram, where α and β are rates of flow:



The quantity of the precursor has been estimated from the kinetics of formation on the basis of several simplifying assumptions. By assuming that all the ultraviolet-absorbing material has the specific activity of the nucleoprotein peak, the amount of P^{32} in the product RNA which is not resolved from the precursor can be calculated. The correction is never large and probably does not seriously influence the conclusion. Figure 27 shows the P^{32} in the precursor and product RNA calculated on this basis as a function of time after addition of the tracer to growing cells.

The curve for the total RNA has the proper shape and magnitude expected from the delay due to the known TCA-soluble pool. By means of a semiempirical curve, shown as the total in figure 27, the two lower curves were calculated by assuming that the precursor contained 10 per cent of the total RNA and that the circulation was negligible ($\beta=0$).

Since the experimental points fit the curves well, it is clear that, if the circulation is indeed negligible, the quantity of precursors is not far different from 10 per cent of the total cellular RNA. If circulation were present the precursor would be larger. The data could not be fitted, however, on the assumption that one base moved from precursor to product for each amino acid incorporated into protein, since β would be greater than 10α .

These findings suggest that the intermediate is RNA of high molecular weight, free or associated with less protein than the bulk of the nucleoprotein. It should be emphasized that neither lipides nor fragments of cell wall or of cell membrane are eluted from the column, and it is observed that a large part of the P^{32} incorporated in short exposures is irreversibly bound to the column. An important step

in the flow of phosphorus into nucleic acids may thereby be missed.

As an interesting sidelight, cells were exposed to chloramphenicol and $P^{32}O_4$ for 2 hours. The ion-exchange analysis shows that no P^{32} was incorporated into the

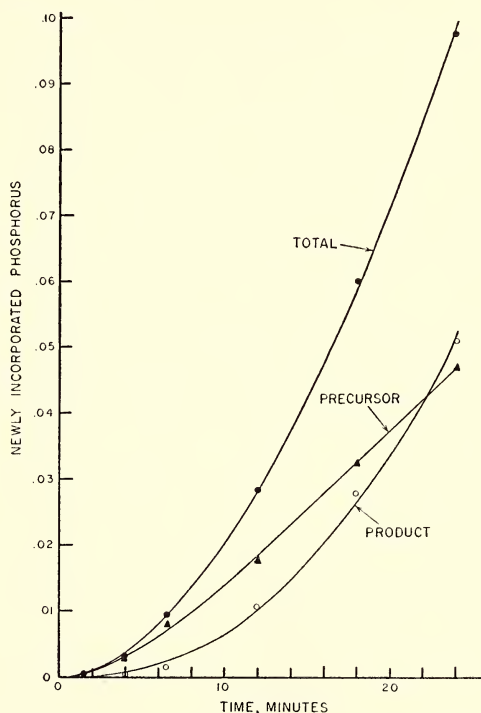


Fig. 27. Time course of P^{32} incorporation into bacterial nucleic acid. The ordinate is the newly incorporated phosphorus expressed as the fraction of the total nucleic acid phosphorus eluted from the column (zero time run). The two curves shown as precursor and product are calculated from the total curve assuming that the amount of precursor is 10 per cent of the product nucleic acid. Cells growing at 37° with a generation time of 69 minutes. Experimental points are shown for the P^{32} incorporated into the total (closed circles), the precursor (triangles), and the product RNA and RNP (open circles).

nucleoprotein peak. The bulk of the P^{32} was eluted at the same salt concentration as the earlier of the precursors described above (fig. 25). In addition, the ultraviolet-absorbing material under the nucleoprotein peak was strongly accentuated, indicating

that RNA having properties similar to those of one of the precursors was piled up in the cell.

VIRUS PURIFICATION

For the past two summers the biophysics group has had one of its members at the Rocky Mountain Laboratory of the U. S. Public Health Service, Hamilton, Montana. The visits have proved mutually beneficial. Studies of interest to the Biophysics Section were carried out with the electron microscope and analytical centrifuge, which were not then available at the Department. At the same time, some of the ion-exchange techniques developed here for study of bacterial components were applied to problems of virology.

In this cooperative venture with Dr. Bill H. Hoyer, of the Rocky Mountain Laboratory, it was found that mammalian viruses and rickettsiae could be purified by means of cellulose ion exchange. During the summer of 1957, poliomyelitis, type 2, virus was grown in human tissue culture cells and purified of host material by a single passage over a bed of the cellulose anion-exchanger "ECTEOLA." A report of this work, which also includes subsequent investigations by Dr. Hoyer and his associates, has already appeared in print (Hoyer, Bill H., Ellis T. Bolton, Richard A. Ormsbee, George LeBouvier, Daniel B. Ritter, and Carl L. Larson, Mammalian viruses and rickettsiae, *Science*, 127, 859-863, 1958).

Two other examples of virus chromatography are shown in figure 28. These chromatograms were obtained as follows: P^{32} -labeled tissue cultures (monkey kidney cells) were allowed to synthesize virus in the presence of P^{32} -labeled orthophosphate. When the host cells had lysed, the radioactive viruses were harvested by ultracentrifugation. The sediment at the bottom of the centrifuge tube was taken up in a small volume of dilute neutral phosphate buffer and loaded onto a column of

ECTEOLA. Buffered salt solutions were then passed over the exchanger, the effluent being collected in separate test tubes at each addition of buffer. The contents of the test tubes were assayed for P^{32} radioactivity and for infectivity. The resulting data are summarized in figure 28. Only a small part of the initial P^{32} was eluted in regions which contained essentially all the viruses that had been loaded onto the exchanger. Protein was barely detectable even by the most sensitive chemical methods available. Thus, a high degree of virus purification could be obtained with great rapidity and relative ease. Such a procedure should prove useful for the production of the purified infectious agents required for careful physicochemical investigations, and also for the production of vaccines free of unwanted host materials, which sometimes induce deleterious "side effects" such as the allergic encephalitis caused by certain vaccines, rabies in particular.

The separation of viruses from host materials, and from one another as figure 28 shows, suggests that the ion-exchange process may also find application in problems requiring virus classification and identification.

The viruses studied to date exhibit a chromatographic behavior differing from that of host nucleic acid, virus nucleic acid, or host nucleoprotein. Since viruses themselves are nucleoproteins, the chromatographic behavior is probably determined by the nature of the protein moiety.

Formaldehyde treatment, as commonly used for the production of vaccines, causes the viral protein to take on a more anionic character than it previously had, and the virus becomes attenuated or even "killed." The feasibility of separating the formaldehyde-modified virus ("dead" ones) from unmodified ("live" ones) by means of ion exchangers has also been demonstrated for polio virus, type 2. In this case formaldehyde-treated viruses are held to the anion exchanger more strongly than untreated

viruses. Thus, the two types can be separated (Hoyer, B. H., R. A. Ormsbee, Ellis T. Bolton, and D. B. Ritter, Demonstration of formaldehyde effect on viruses, *Federation Proc.*, 17, 517, 1958). Although

INCORPORATION OF AMINO ACID ANALOGS INTO BACTERIAL PROTEINS

Considerable quantities of certain amino acid analogs may be incorporated into the proteins of *Escherichia coli*. The analogs

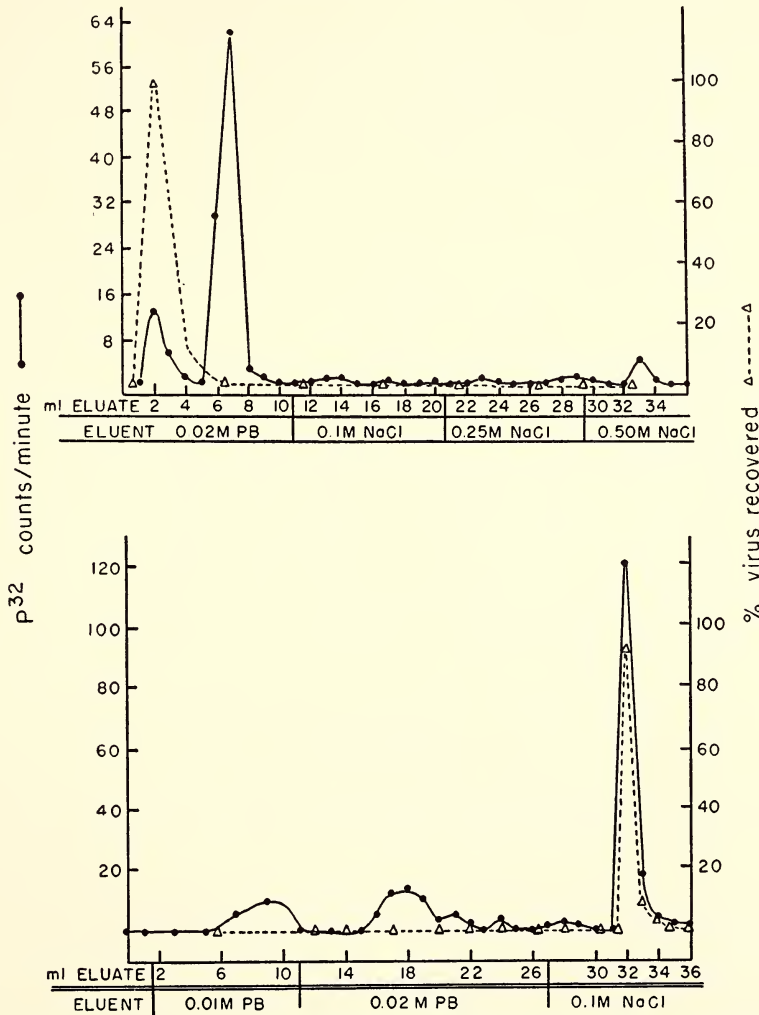


Fig. 28. Chromatography of animal viruses on ECTEOLA-cellulose. The viruses were labeled with P^{32} . Each tube of effluent was assayed for radioactivity and infectivity.

it is too early to judge whether the ion-exchange method will contribute in a practical way toward the production of safe vaccines, its simplicity, economy, and efficiency recommend it as an adjunct to the biologists' tools for the production of highly purified infectious agents.

substitute for corresponding naturally occurring amino acids and cause various biological effects. In general, cellular growth becomes linear, and specific enzymatic functions may be lost, be suppressed, or remain unaffected. Such effects depend upon the degree and kind of sub-

stitution produced. Since the degree and kind of substitution can be controlled, analogs provide a means for the quantitative examination of the relationship between altered molecular structure and enzymatic activity. Evidence can also be adduced concerning susceptibility of bacterial protein types to analog substitution.

The demonstration that amino acid analogs could be incorporated into bacterial proteins immediately raised many ques-

The analog, *norleucine*, substitutes for methionine in the proteins of *E. coli*. A reduction of 38 per cent of the protein methionine is obtained when the methionine-requiring mutant (ML 304d) is grown in C medium containing DL-norleucine ($2 \times 10^{-2} M$) and S^{35} L-methionine ($10^{-4} M$). This mutant was chosen in order to eliminate competitive reactions involving sulfur compounds other than methionine or the methionine analog. The

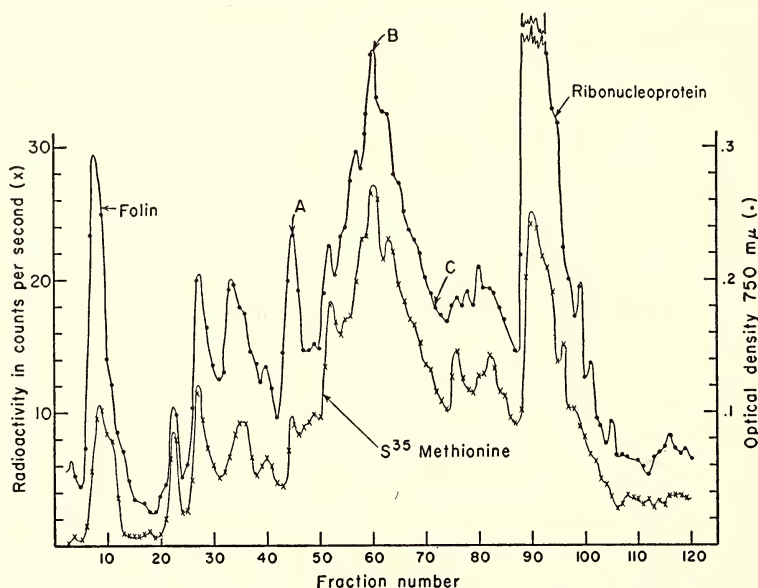


Fig. 29. Elution pattern of bacterial extract of *E. coli* obtained with a DEAE ion-exchange column. Mutant cells (ML 304d) grown in C medium containing S^{35} L-methionine ($10^{-3} M$), thiomethyl β -D-galactoside ($5 \times 10^{-4} M$), and maltose.

tions about the nature of the proteins produced. In collaboration with Dr. Georges N. Cohen, of the Pasteur Institute, Paris, France, and H. de Robichon-Szulmajster, National Institutes of Health, U.S.P.H.S., Bethesda, Maryland, investigations were carried out to determine whether the analogs are contained in radically different molecular species or in proteins similar to those normally synthesized. These investigations required: (a) an analog that would substitute for only one naturally occurring amino acid, and (b) a quantitative method for analyzing bacterial proteins.

separation of bacterial proteins into chromatographically resolvable "protein classes" was achieved through the use of the DEAE-cellulose ion-exchange column. Figure 29 shows the elution pattern of an extract of *E. coli* grown in C medium containing S^{35} methionine. Thiomethyl β -D-galactoside was added to induce the synthesis of β -galactosidase.

The bacterial extract was prepared from washed cells, ruptured by extrusion through a small orifice under pressure, the extruded material being centrifuged to remove whole cells and large cellular fragments. The opalescent supernatant was

then used for the column analysis. Evident in this elution diagram are a number of well resolved regions showing a close correlation between the protein pattern (measured by the Folin reagent) and the pattern of distribution of the radiomethionine. Two-dimensional paper chromatograms of hydrolysates of an aliquot of the bacterial extract showed that the incorporated radioactivity was contained solely as methionine.

In figure 30 are shown the specific radio-

are, of course, contained in this region. Nevertheless, the partitioning of bacterial proteins into protein classes is apparent.

Column analysis of bacterial extracts of cells grown in C medium containing DL-norleucine ($2 \times 10^{-2} M$) and S^{35} L-methionine ($10^{-4} M$) gave elution patterns similar to figure 29. A significant difference was a *uniform reduction* in the specific radioactivities of these bacterial proteins compared with those of the control experiment

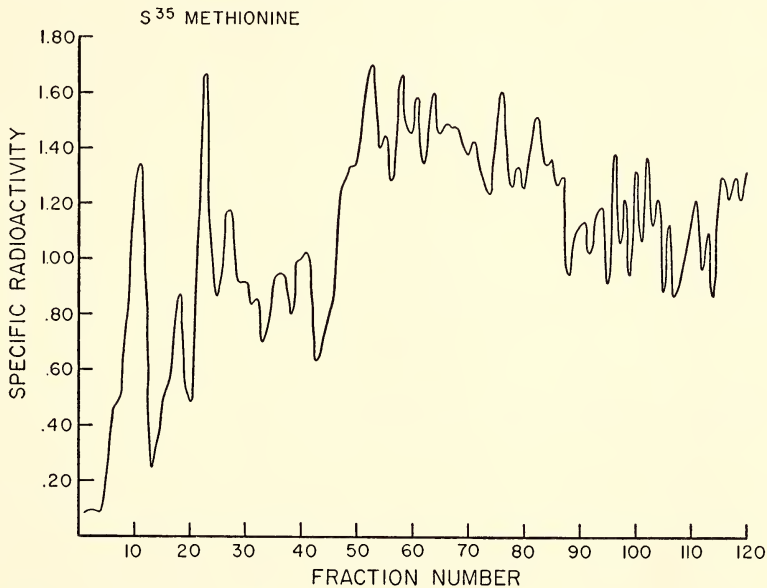


Fig. 30. Specific radioactivity of eluted column fractions. Data represent the ratio of radioactivity per fraction to the quantity of protein newly synthesized after the addition of the labeled methionine to the culture.

activities of the individual fractions. The specific radioactivity is the ratio of the quantity of radioactivity to the amount of newly synthesized proteins. This figure demonstrates that the methionine content varies among the protein classes resolved.

Figure 31 shows the degree of resolution among the eluted proteins. Superimposed on the elution diagram are the locations of three enzyme activities: β -galactosidase (LAC), phosphoglucomutase (MUT), and glucose-6-phosphate dehydrogenase (ZW). Each enzyme activity is correlated with a well resolved protein peak. Other proteins having similar charge properties

(fig. 17). The existence of certain markers (peaks, valleys, enzymes, etc.) along the elution diagram allows a quantitative comparison, marker for marker, among several column runs. Figure 32 shows the specific radioactivities of seven well marked and separated regions obtained with the norleucine-grown cells. These are compared with the same regions in the control experiment, where the specific radioactivity of each region was arbitrarily chosen to equal 100.

The regions compared in figure 19 were: two well resolved and isolated protein peaks *A* and *B*, the ribonucleoprotein

peak, the three peaks of enzyme activity (LAC, MUT, and ZW) easily measurable in both experiments, and region C shown in figure 16. Each point represents the

arithmetical mean of the specific radioactivity of the maximum peak sample and the two samples immediately preceding and following this peak.

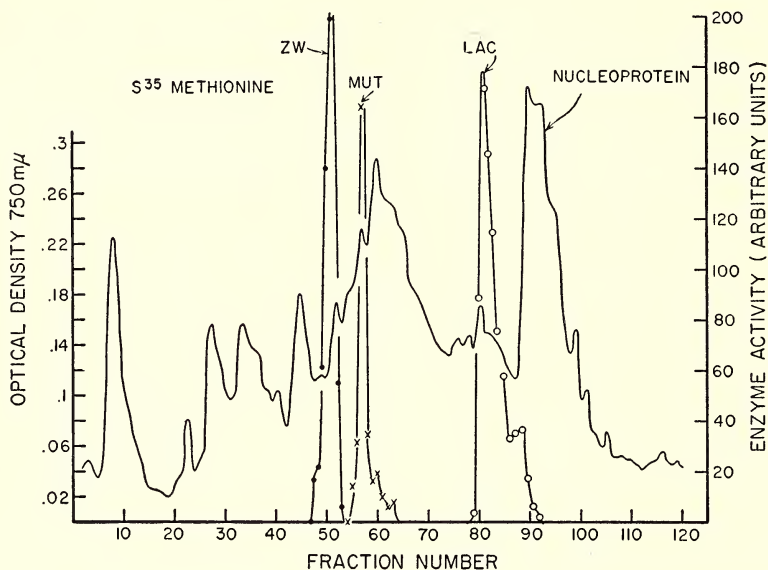


Fig. 31. Location of enzyme activities along elution diagram. Glucose-6-phosphate dehydrogenase (ZW); phosphoglucomutase (MUT); β -galactosidase (LAC).

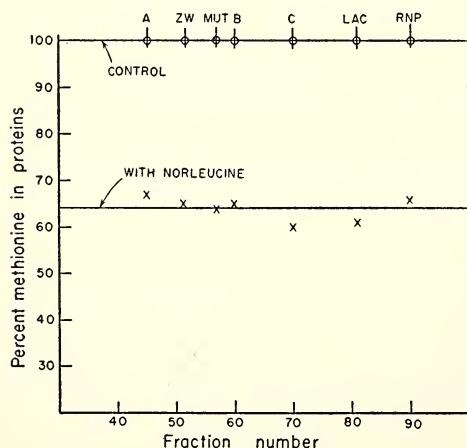


Fig. 32. Comparison of specific radioactivities of definite regions along elution diagrams obtained from cells grown in C medium containing S^{35} L-methionine (10^{-3} M) (control) and from cells grown in C medium containing S^{35} L-methionine (10^{-4} M) plus DL-norleucine (2×10^{-2} M). Linear growth was obtained in the latter culture, and the cells were harvested for analysis after more than a doubling of bacterial mass.

Figure 33 shows an elution pattern obtained from cells grown in DL-norleucine $1-C^{14}$ (2×10^{-2} M) and nonradioactive L-methionine (10^{-4} M). In this experiment there was a 43 per cent substitution of norleucine for methionine in the bacterial proteins. Radioautographs of two-dimensional paper chromatograms of hydrolysates of the bacterial extract showed one radioactive, ninhydrin-positive spot having the same R_f as found with the labeled norleucine used in this experiment.

There is a great deal of similarity in the elution diagrams obtained from the S^{35} methionine and the C^{14} norleucine labeled cells. One significant difference, however, occurs in the first major peak of the elution diagrams. In these early fractions of eluted material are contained the nonprotein amino acids (or analogs) concentrated by the cell from the environment. The quantity of "free amino acids" depends upon their external concentrations, and in these

experiments the ratio of C^{14} L-norleucine to S^{35} L-methionine in the media was 100 to 1. Chemical fractionation of the eluted fractions showed that TCA-soluble material ("free amino acids") was mainly contained in the first 20 samples and dropped rapidly to a few per cent by the thirty-fifth sample, remaining low for the rest of the elution process. It has also been

nine and norleucine (figs. 29 and 33) also eliminates the hypothesis that only certain proteins are susceptible to analog substitution. Indeed, figure 32 demonstrates that the analog is incorporated into *all* the proteins and *in the same proportion*. Each methionine incorporation site thus seems to have an equal probability of analog substitution. The formation of a large

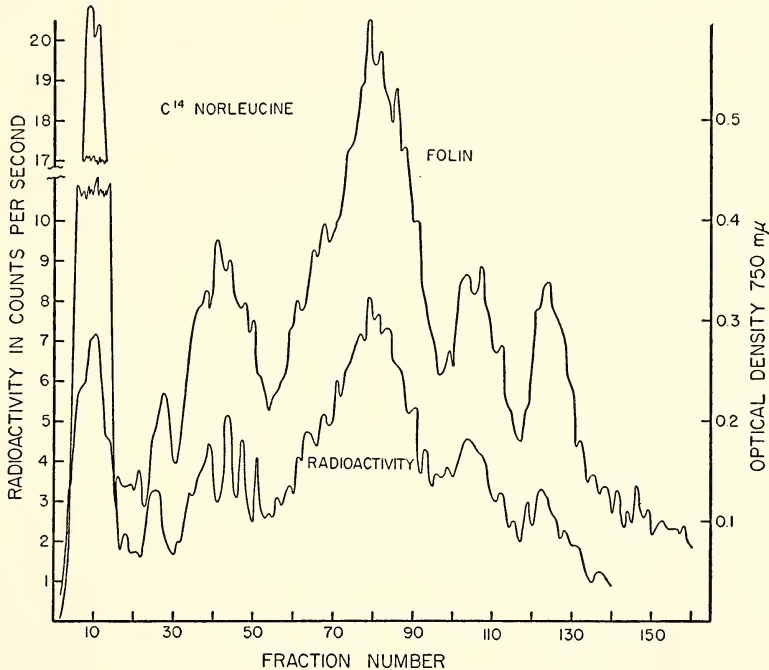


Fig. 33. Elution pattern of bacterial extract of 1- C^{14} DL-norleucine-grown cells. Mutant cells (ML 304d) grown for more than a doubling in C medium containing labeled norleucine ($2 \times 10^{-4} M$) and S^{32} L-methionine ($10^{-4} M$).

noted that the quantity of material contained in the ribonucleoprotein region varies from one column run to another, and, if the cells are ruptured in media containing phosphate buffer, or in buffer containing no magnesium, this ribonucleoprotein is not seen at all.

The above results demonstrate that most of the proteins formed in the presence of the analog are not radically different molecular species but are physicochemically similar to the proteins normally synthesized. The similarity of the elution diagrams obtained with the labeled methio-

quantity of uncompleted proteins, caused by the joining of the analog by a peptide bond to one of its neighboring amino acids but not to the other, does not seem to be a probable event. Should such unfinished molecules be present, they would markedly alter the elution patterns obtained after the analog is incorporated.

These conclusions are strengthened by data obtained in experiments using other amino acid analogs. Figure 34 shows the elution diagram obtained from wild-type *E. coli* (ML 30) grown in C medium containing C^{14} 3-DL-phenylalanine ($10^{-4} M$)

and DL-*p*-fluorophenylalanine (5×10^{-3} M). At these concentrations there is approximately a 50 per cent substitution of the analog for protein phenylalanine, and linear growth occurs. The elution diagram obtained (fig. 34) appears very similar to that from normal cells (fig. 29). There is no evidence of different types of protein classes being formed as a result of analog substitution.

Figure 35 demonstrates, within the limits of resolution of the column and a low

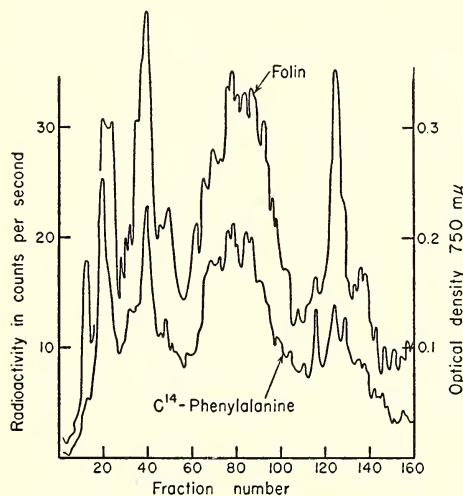


Fig. 34. Elution pattern of an extract of cells grown in the presence of C^{14} -3-DL-phenylalanine and DL-*p*-fluorophenylalanine.

specific radioactivity of tracer, that *p*-fluorophenylalanine is incorporated into all the bacterial proteins. This elution diagram was obtained from wild-type *E. coli* grown in C medium containing C^{14} 3-DL-*p*-fluorophenylalanine (5×10^{-3} M).

The use of another amino acid analog gave results which in every respect confirm and augment the conclusions cited above. Selenomethionine *completely* substitutes for the methionine of the bacterial protein. With this *uniform* replacement exponential growth was observed and the induction and synthesis of active β -galactosidase demonstrated. The constitutive enzymes essential for exponential growth were obviously present in active forms. Under these

conditions there can be little doubt that active *altered* proteins are synthesized, having biological as well as physicochemical properties similar to those of the normal cell.

The use of amino acid analogs other than selenomethionine has always resulted in linear growth of the cells whenever analog substitution in the protein was evident. Thus, it might be argued that at least one growth-rate-limiting enzyme was unusually susceptible to analog substitution and that the enzyme was synthesized at a reduced rate if at all. On the other

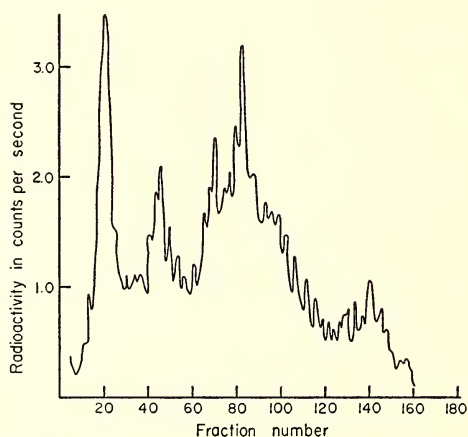


Fig. 35. Elution pattern of an extract of cells grown in the presence of C^{14} -3-DL-*p*-fluorophenylalanine.

hand, analog incorporation might result in the synthesis of protein molecules—but these would be proteins without the capacity for enzymatic function. This elimination or suppression of enzyme activity would depend on the degree and kind of substitution involved and on the amino acid composition of the sites of enzyme action. Some evidence supporting the latter hypothesis has accumulated, and the investigation of this question is currently under way.

CELL-FREE SYSTEMS

Systems free of complete, undamaged cells have frequently been tested for their ability to synthesize protein. One goal in

using such systems is to find the minimum number of carefully purified and characterized components necessary for protein synthesis. A much more limited endeavor is the use of fractured cells in which the components may be selectively destroyed by enzymes. A number of these systems have been tested in this laboratory by means of radioactive-tracer techniques, which are extremely sensitive. Unfortunately, we have not yet found any cell-free system that gives reproducible and convincing incorporation of amino acids into protein.

Various mixtures of purified ribosomes with soluble proteins were tested. These preparations could be made assuredly cell-free by repeated high-speed centrifugation. No sign of incorporation of amino acids of high specific radioactivity (12 per cent C^{14}) was observed in any of the tests. When cell walls were added to the mixtures, or tested alone, incorporation was observed. Furthermore, it was the large fragments of cell walls that gave the greatest incorporation. In these preparations whole cells were not readily observable in the wet material under the phase contrast microscope. Nevertheless, slides stained with gentian violet to show whole cells invariably revealed their presence in sufficient quantity to account for the observed incorporation.

In last year's report the formation of "protomorphs"—large globular structures that form in clear solutions of cell extracts—was described. Their formation and their incorporation of amino acids were erratic. During this report year some of the causes of variation have been determined. The formation process is sensitive to concentration. A twofold dilution of the usual pressure cell juice is sufficient to prevent formation, but cell juices prepared by grinding or other processes that preserve intact DNA will form protomorphs from much more dilute solutions. Furthermore, the addition of DNAase to the solution invariably prevents protomorph formation. DNA is one of the com-

ponents of protomorphs, and it seems to be an essential one. Phosphate is also essential. When the cells are very thoroughly washed, protomorphs will not form unless PO_4 is added back to 10^{-3} M. The quantity of intact DNA could easily vary, depending on the conditions in the pressure-cell orifice, and the quantity of PO_4 could vary with the conditions of washing. These factors seem to be sufficient to account for the observed variations in formation of protomorphs.

The incorporation of amino acids by protomorphs was at times convincing. In particular, the incorporation increased with increased concentration of amino acid or upon supplementation with ATP (conditions that should not affect intact cells). Also, the pattern of amino acid incorporation did not resemble that of whole cells.

Unfortunately, the incorporation by the protomorphs was also highly erratic. One series of tests showed that the capacity of protomorphs to incorporate amino acids depended on the presence of cell-wall fragments. Again it seems possible to ascribe the observed incorporation to whole cells hidden from view by the protomorphs.

As a consequence of these equivocal results, work with cell-free systems has proceeded only occasionally. The cell wall or cell membrane appears to be the most likely fragment to show true synthetic activity, but it also is the most likely to be contaminated by intact cells. The goal of reconstituting a synthesizing system from purified components still seems very remote.

THE METABOLIC POOL PROBLEM IN *E. COLI*

For several years the ability of *E. coli* to concentrate low-molecular-weight compounds has been the subject of intensive study. In the past year our experimental activities shifted to other aspects of the mechanisms of macromolecular synthesis. Measurements of the exchange rate of the proline pool at 0° C provide the only new experimental information, but the impli-

cations of the large body of previously obtained experimental results for the models of the pool-forming process are now fairly well understood. It has become clear that neither the simple "permease" model nor the "stoichiometric site" hypothesis is sufficient. A model in which a limited quantity of carriers is utilized to place the pool compounds on sites appears, however, to be sufficient to explain practically all the observations.

Exchange Rate of the Proline Pool at 0° C

Observations of exchange between amino acids present externally and those in the pool can be conveniently carried out under a variety of conditions by means of labeled compounds. If they are carried out at the normal temperatures used for growth and pool formation (20° to 40° C) the rates of pool formation, protein incorporation, and exchange are all comparable. Also, in quasi steady-state conditions, the pool size and external concentration cannot be varied independently. At 0° C, however, the pool formation and protein incorporation rates are very much more strongly suppressed than the exchange rate (as reported in Year Book 56). As a result the pool size will not show significant change in several hours, even when it is not in equilibrium with the external concentration, and this is sufficient time for exchange equilibrium to occur between the external and pool amino acids. Thus, exchange studies can be carried out over a wide variety of independently varied concentrations and pool sizes.

Since pool formation is very slow at 0° C, pools of a desired size are formed with unlabeled proline at 25°. The suspension is then chilled to 0° C. The cells are centrifuged and resuspended in buffer, and after an hour or so at 0° C, C^{14} proline is added. Since the external quantity is small compared with the amount in the pool, a very efficient labeling of the pool by exchange is achieved. After a steady state (equal internal and external specific activities) is reached, the external concen-

tration is brought up to a chosen value by adding C^{12} proline. The time course of exchange is then followed by measuring the loss of TCA-soluble radioactivity from the cells. Typical measurements under these conditions are shown in the curve of figure 36.

When the results of such experiments over a wide range of concentrations and pool sizes are examined it is found that the time course of exchange can be re-

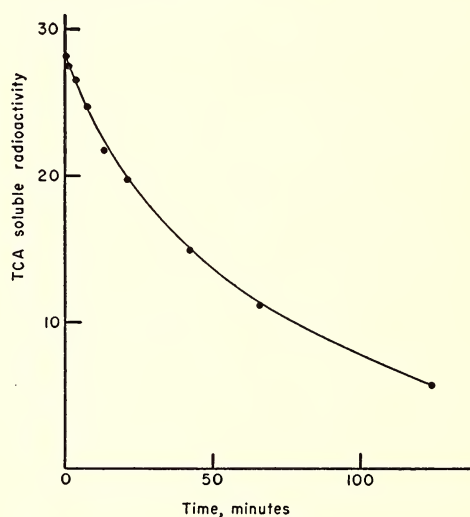


Fig. 36. Time course of exchange of the proline pool at 0° C. Cells with a C^{14} proline pool at 1.5×10^{-6} mole per wet gram were prepared as described in the text. At time zero, C^{12} proline was added to a concentration of 7×10^{-4} M.

solved into two components with widely different time constants. Figure 37 shows the dependence of the rate of exchange calculated for each of these components as a function of the pool size. The figure beside each point is the external concentration during exchange in micromoles per liter. It appears that the exchange rate is independent of the external concentration except possibly at low concentrations.

The rapidly exchanging component of the pool is always smaller than the slowly exchanging one. It appears to saturate at less than 1.0 micromole per gram wet cells and is not easily observable when the total pool is greater than 10 micromoles per

gram. Such exchange experiments are difficult to perform since the larger pools appear to be unstable at 0° C.

The exchange rate of the large, slow component is roughly proportional to the total pool size. Unfortunately, the accuracy of the data is not quite sufficient to determine whether the exchange rate of

owing to the simplicity of exchange processes. If a single homogeneous component of the pool exchanges with external amino acid, the time course of the process must follow a simple exponential represented by a single time constant. This statement holds, regardless of the nature and multiplicity of the mechanisms mediating the

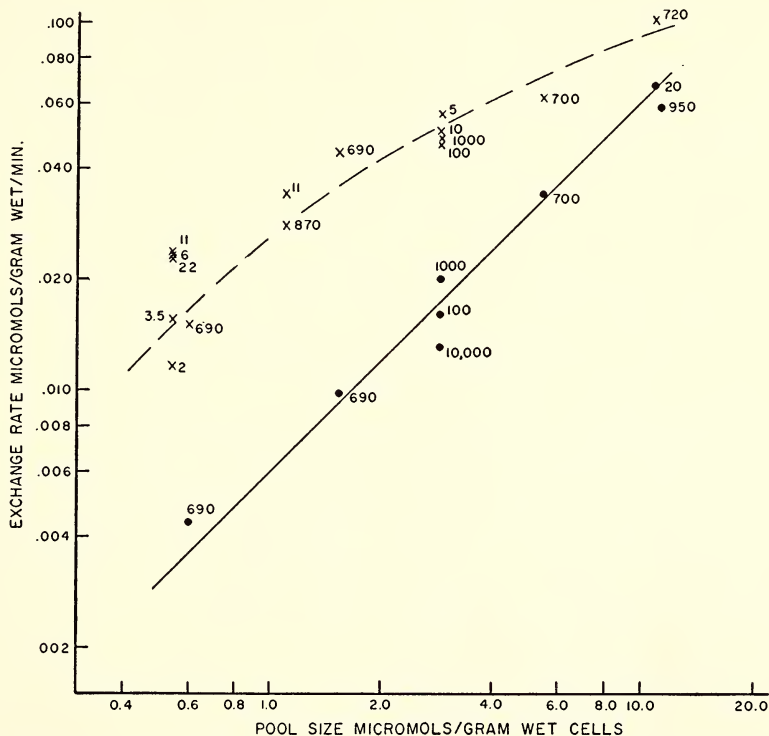


Fig. 37. Rate of exchange as a function of pool size (log log plot). The points were obtained from experiments such as that shown in figure 14 by fitting the time course of exchange to curves derived from the sum of two exponential decays. The numbers beside each point are the external concentrations during exchange in micromoles per liter. The straight line shown would result if the exchange rate were proportional to pool size.

each of the components is proportional to its own size, although this result is suggested by the evidence.

In addition, it is not certain that there are only two components, although there are clearly more than one, since none of the exchange curves fits a simple exponential. These results show that the proline of the pool is held in the cell by more than one mechanism, or at least with varying degrees of affinity.

The evidence for this statement is good,

exchange, as long as the quantity of amino acid associated with intermediate steps is negligibly small compared with the size of the pool. The significance of these results will be discussed below.

Description of Models

Figures 38 and 39 describe three models for the pool-forming process. The first (fig. 38) is more or less equivalent to the "permease" model suggested by Monod and Cohen. It is assumed that there is an

association of the external amino acid with a specific transport mechanism. This mechanism, being coupled with a source of energy, can form an association with the amino acid in the dilute external medium and then release it into a region

site-amino acid complex. The pool saturates when all the sites for the particular amino acid are occupied. The process of dissociation of the site must also be coupled to the energy supply since the pools are maintained in the absence of an energy

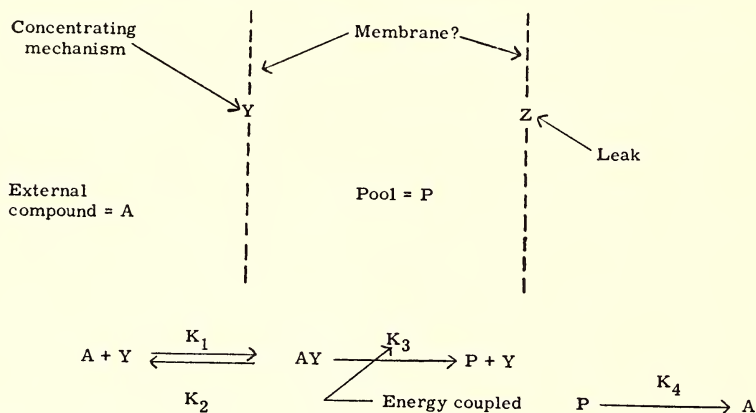


Fig. 38. Permease model.

of high concentration within the cell. In order that the pool size saturate at high external concentration it is assumed that a separate mechanism allows the pool compound to leak out of the cell. Thus at high external concentrations the concentrating mechanism becomes saturated. When its maximal rate is balanced by the leak rate the pool can no longer be increased. According to the permease model the pool is maintained by means of a circulating flow—a sort of dynamic steady state. This feature is borne out by experiments showing rapid exchange during pool formation.

The upper model indicated in figure 39 is the simplest site model, referred to by Monod and Cohen as the "stoichiometric site hypothesis." It is assumed that, by means of an unspecified mechanism coupled to the energy supply of the cell, free amino acid diffusing through the cell is caused to form a labile bond with a specific site. The relationship between the pool size and the external concentration is maintained by a balance between the rates of formation and dissociation of the

supply (although they are not formed under these conditions). It must be assumed that exchange occurs by a separate process.

The lower model shown in figure 39 is entitled the "site plus carrier," since a

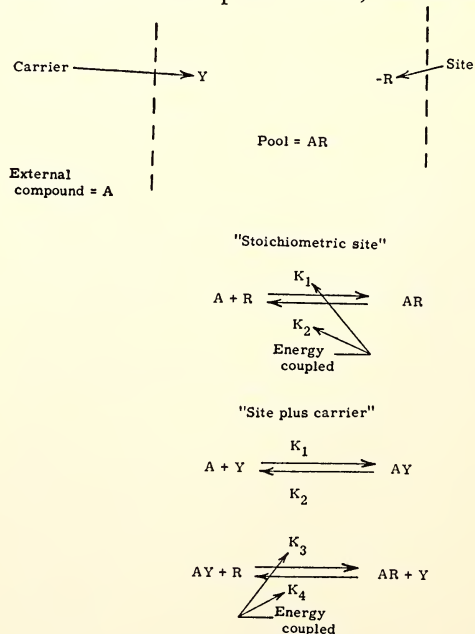
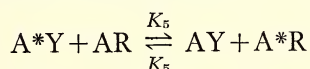


Fig. 39. Site models.

carrier has been postulated in order to account for a wide variety of observations of the pool-forming process. A carrier-amino acid complex is formed without the participation of the cell's energy-supplying mechanisms. The carrier complex (AY) then reacts with a free site to form a site complex (AR) and free the carrier. The free carrier may in turn react with the site complex in the reverse reaction and form AY. Both these reactions must be coupled to the energy supply of the cell in order that the pool be maintained in the absence of an energy supply. A given carrier is present in limited quantity and is specific for a particular amino acid. The carriers and carrier-amino acid complexes must be free to diffuse through the cell in order to reach and react with the sites or site complexes. It is also presumed that in the presence or absence of an energy supply an exchange reaction occurs:



The carrier indeed carries a heavy burden of properties that may be conceptually difficult. Nevertheless the resulting model is simple enough to be mathematically analyzed in detail and predicts the principal properties of the pool.

Summary of Pool Properties and Implications of the Models

Table 8 lists in summary form the principal observations of the behavior of the proline pool. In the columns at the right the score for each of the models is listed, a plus sign indicating that the model satisfactorily explains the observation and a minus sign that there is a contradiction or that a modification may be required by the experimental evidence.

Since most of these observations have been discussed in the annual reports of previous years, no attempt will be made here to follow in detail the arguments leading to these scores, except for the new evidence concerning the exchange rate at 0° C.

As has already been mentioned, the 0° exchange data show that there is more than one component in the proline pool. Items 13 and 16 of table 8 also indicate that there are different mechanisms for the maintenance of compounds in the pool. This evidence may be incorporated in the site models by assuming that there are sites of different types or affinities within the cell.

In order to incorporate this evidence into the permease model it might be assumed that there are internal regions within the cell having different degrees of accessibility to the external amino acid (to explain the exchange data) and different sensitivities to osmotic shock. Alternatively, it might be assumed that sites within the cell retain part of the pool while the entry mechanism is still that of the permease model.

If the arguments are followed in detail it is found that the addition of internal sites to the permease model does not answer the difficulties arising from items 2, 3, 4, and 7 of table 8, although it does help as far as items 8 and 16 are concerned. The very existence of a high rate of exchange (when pools can only be formed slowly) raises serious difficulties for the permease model (items 3, 4, and 7). It would be necessary to specify what features would be added to this model to allow for exchange and maintenance before the rates of exchange could be discussed.

The experimental results of Cohen and Monod at the Institut Pasteur on the galactoside-concentrating system of *E. coli* are almost entirely consistent with the permease model. In fact, it was these results that led them to postulate the model. They do, however, report one observation which has a similar consequence to that of items 7 and 4 of table 8. The concentration of galactosides is blocked by the lack of an energy source or by azide. But in whole cells the rate of splitting of orthonitrophenylgalactoside by the internal enzyme β -galactosidase is not reduced under either of these conditions. Thus, although under

these conditions pools may not be formed, galactosides may enter the cell at a rapid rate when the energy supply for the "Y mechanism" of the permease model has been blocked.

Both the site models predict that the exchange rate should be proportional to the

The quantitative observations corresponding to items 10, 11, and 12, and the relationship between pool size and external concentration, supply five independent relations between the constants of the "site plus carrier" model. The total number of sites is known from the saturation value

TABLE 8. Relation of Observations of the Proline Pool of *E. coli* to the Models

Observation	Score		
	"Permease"	"Stoichiometric Site"	"Site plus Carrier"
1 Glucose required for formation	+	+	+
2 Glucose not required for maintenance	—	+	+
3 Pools maintained at 0° C	—	+	+
4 Pools formed slowly at 0° C	—	+	+
5 Pools may be very large	+	—	—
6 Exchange between external and pool AA occurs during formation	+	+	+
7 Exchange occurs at a high rate in absence of glucose or at 0° C	—	+	+
8 Fast and slow components appear in exchange curves at 0° C	—	+	+
9 The 0° C exchange rate saturates at low external concentration	?	—	+
10 The 0° C exchange rate increases with pool size	?	+	+
11 Rate of loss from pool in absence of external AA is slower than initial rate of formation	—	—	+
12 Initial rate of pool formation does not rise as fast with concentration as pool size does	—	—	+
13 Small pools not generally influenced by other AA but large pools are suppressed	—	+	+
14 Pools removed by sudden reduction in osmotic strength	+	+	+
15 Pools may be immediately re-formed after removal by shock	+	+	+
16 Different pools removed to different extent by shock	—	+	+
17 Maximum pool size increases with osmotic strength of medium	—	—	—

* Assuming that the sites may be osmotically sensitive.

pool size. However, the limited quantity of carrier postulated in the "site plus carrier" model is necessary in order that the exchange rate be independent of the external concentration, for moderate concentrations. The limited quantity of carrier plays a role in forcing the predictions of the model to match items 11 and 12 of table 8. It is also useful in understanding the interactions between similar amino acids reported in Year Book 55.

of the pool. From these relations the constants K_1 , K_2 , K_3 , K_4 , and K_5 have been numerically evaluated, except for a constant factor which is the reciprocal of the total number of carriers present. With a reasonable assumption as to the number of carriers, all the constants are consistent with principles of chemical kinetics.

Thus, we have been able to explain the wide variety of observations concerning the pool and to approach an evaluation of

the reaction rate constants of the postulated steps. The equations which in the end represent the pool-forming process will have to be similar to those derived from this model. It is always possible, however, that they could be derived from a fundamentally different model. Thus, the existence of the carrier has not been directly demonstrated although there is a requirement for some equivalent mechanism to account for the limited rates of formation and exchange.

Such a carrier may also be important in protein synthesis since it would be a suitable specific mechanism for the transport of amino acids from the pool sites to sites that are actively engaged in that process.

SYNTHESIS IN MOUSE TISSUE

During the year our collaborative work with Drs. L. B. Flexner and J. B. Flexner on protein synthesis in mouse tissues has continued. These experiments provide the Biophysics Section with an opportunity to maintain a working knowledge of mammalian cells. It is of the greatest interest to observe the many ways in which these cells resemble microorganisms and others in which there are notable differences.

The absolute quantity of pool amino acids in the liver and cerebral cortex of both newborn and adult mice was determined (table 9). In contrast to the tissue proteins, where there is little difference in amino acid composition between adult and newborn, the amino acid pools change markedly with maturation. These values are needed to calculate the flux of amino acids from pool to protein.

Figure 40 shows the passage of radioactive glutamic acid from blood plasma to tissue pool to tissue protein in the adult mouse. The rapid transfer from plasma to cortex is of particular interest because the blood-brain barrier has previously been presumed to be impermeable to glutamic acid in the adult. Similar observations were made on the transfer of a group of essential amino acids and the utilization of

glucose for synthesis of nonessential amino acids.

The rates at which individual amino acids are transferred to protein were then calculated. If amino acids were incorporated into protein solely as the result of synthesis of a complete molecule, the quantity of amino acid incorporated should be proportional to the quantity in the protein. On the other hand, if amino acids were incorporated by exchange into pre-existing protein, the quantity should depend only on the exchange rate. The observed rates of incorporation of different amino acids are by no means proportional to their concentrations in the protein. Moreover, maturation has different effects on the rate of incorporation of different amino acids. For some, the incorporation rate is higher in the newborn than in the adult; for others, it is equal or less in spite of the close correspondence of the amino acid composition of the protein at the two ages.

Although such results suggest that exchange plays an important part in the incorporation of amino acids by mammalian cells, there is an alternative interpretation. Only the average composition of all the protein of the tissue was measured, and the incorporation rate observed was an average rate. It is, then, quite possible that certain proteins of the tissue, which are synthesized and degraded rapidly, account for most of the incorporation, whereas the average composition is determined by other protein.

This uncertainty can be resolved by measuring the compositions and incorporation rates for individual isolated proteins. In addition, it is of considerable interest to observe whether there is a range of stability among the proteins of a tissue. Accordingly, some exploratory experiments have been made using the DEAE columns described above to separate out individual proteins from different tissues of the mouse.

For these experiments a much higher level of radioactivity was needed to ob-

serve individual proteins instead of the total. S³⁵O₄ (25 millicuries) was supplied to a culture of *E. coli* which converted roughly 60 per cent to S³⁵-labeled cystine and methionine of the cells. The cells

placed on the column and eluted with a salt gradient. The different tissues (liver, cortex, muscle, tumor) gave different elution patterns, each one showing at least four resolved protein peaks. The radioac-

TABLE 9. Amino Acid Composition of Tissue Pools and of Tissue Protein

Results on the amino acid pools are expressed in terms of the mean and its standard error. Number of samples is in parentheses. Since glutamine is lost on the column the observed values are falsely low. The value for glutamic acid in protein includes glutamine converted to glutamic acid by acid hydrolysis. Ph. al. + = phenylalanine + leucine + isoleucine.

Amino Acid	μg in pool/100 mg tissue				mg/100 mg protein			
	Liver		Cortex		Liver		Cortex	
	Newborn	Adult	Newborn	Adult	Newborn	Adult	Newborn	Adult
Aspartic	22 ±3.5(5)	6.3±1.0(6)	20 ±1.6(6)	31 ±3.0(7)	10 (1)	10 (1)	11 (1)	11 (1)
Glutamic	28 ±3.6(5)	26 ±1.9(6)	47 ±4.2(6)	110 ±5.8(7)	10 (1)	9 (1)	16 (1)	17 (1)
Glycine	13 ±1.1(6)	7.0±0.7(6)	10 ±1.1(6)	6.3±0.4(7)	4 (1)	4 (1)	3 (1)	3 (1)
Serine	9.0±0.8(6)	4.8±0.3(6)	7.0±0.8(6)	4.4±0.4(7)	4 (1)	5 (1)	4 (1)	3 (1)
Alanine	13 ±1.2(6)	20 ±1.7(6)	9.0±1.2(6)	7.0±0.9(7)	5 (1)	6 (1)	5 (1)	5 (1)
Ph. al. +	8.1±0.9(6)	6.0±0.8(5)	6.2±0.7(6)	2.5±0.3(7)	24 (1)	21 (1)	14 (1)	17 (1)
Glutamine	>10 ±1.7(5)	>19 ±4.0(4)	>13 ±2.4(4)	>22 ±4.8(6)				

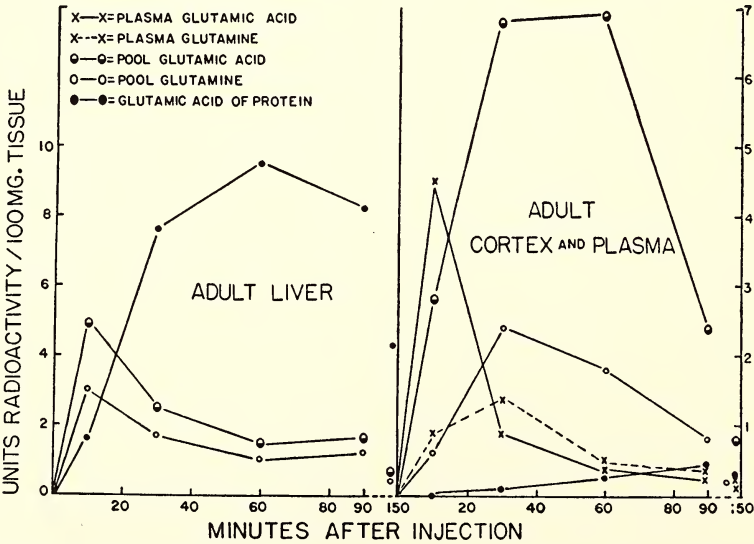


Fig. 40. Radioactivity of glutamic acid and glutamine in blood plasma and in amino acid pools of cortex and liver, and of glutamic acid in proteins of cortex and liver, after subcutaneous injection of L-glutamic acid randomly labeled with C¹⁴.

were harvested, washed, and hydrolyzed, and hydrolysate was injected into two mice. One mouse was killed after an hour, the other after 2 hours. Various tissues were dissected out and homogenized, and the cells were broken with the pressure cell. Samples were then

tivity per unit protein varied considerably from one peak to another, indicating either a difference in the sulfur content or a difference in the rate of incorporation in the different proteins. Even this single exploratory experiment showed unequivocally that there is no single protein con-

stituent of the brain cortex which accounts for most of the incorporation.

In the same experiment 8-m μ slices were made of the cortex. Radioautographs of the slides, compared with the same section stained with thionine seen visually, show that it is largely the cell bodies of the brain and not the nerve processes that incorporate S³⁵ into protein (fig. 41, pl. 3). From the intensity obtained it seems possible that useful radioautographs could be made after exposures to the tracer of 5 minutes or less. In such short times it might be possible to correlate the areas of the brain that synthesize proteins with different functional states of the animal.

HYDRA AND PLANARIA

In Year Book 55, some advantages of using relatively simple multicellular animals for investigations of growth, differentiation, and reproduction were discussed and preliminary studies with radioactive substrates were reported. Further investigations on *Hydra* and some preliminary studies on *Planaria* have been carried out during the past year.

Feeding and Digestion

Because of the relative impermeability of *Hydra* to free substrates, it was found more efficient to feed radioactive tissues to the hydras. The animals were fed small bits of S³⁵-labeled mouse lung from the carcass of a mouse used in the experiments carried out in collaboration with Dr. L. Flexner, as described elsewhere in this report. Each piece of sulfur-labeled tissue was soaked in 10⁻⁴ M glutathione (GSH) for 5 minutes and, with the aid of watchmaker's forceps, placed individually in the center of the ringlet of tentacles near the hypostome. As demonstrated by W. F. Loomis, a hydra will swallow nearly any solid object of acceptable dimensions in the presence of GSH. With very little practice the investigator was able to feed about 50 hydras in a half hour.

Usually 10 to 12 S³⁵-labeled hydras were

sufficient for chemical fractionation. The animals were washed, placed in 0.2 ml distilled water, and disrupted by sonication. The TCA-soluble fraction was filtered; the activity of the remaining particles was considered in the protein calculations. In addition, the TCA-precipitable alcohol-soluble fraction was separated from the TCA-precipitable alcohol-insoluble fraction by filtration; the latter fraction was counted directly on the dry filter. Experiments performed during the first 6 hours after ingestion required measurements of the radioactivity of the food within the hydra's tissues and of the ungested food within the gastrovascular cavity. The hydras were bisected longitudinally with a scalpel and washed free of all visible material as discerned using the dissecting microscope. The hydra tissue, free of cavity contents, was then fractionated; the washings were saved and counted.

The hydra has a large gastrovascular cavity in which its solid food is broken into pieces small enough to be engulfed by endodermal cells. Upon engulfment digestion continues intracellularly. This was demonstrated by cutting the animal open, washing it free of all visible food, and chemically fractionating it. The food not engulfed was also fractionated. The relative proportions of the TCA-soluble and -insoluble fractions of the hydra were similar to those of the ingested food (cf. fig. 42). Examination of the alcohol-soluble (AS) and alcohol-insoluble (AI) fractions of the hydra, however, revealed that a change in the proportions of these radioactive components occurred between 1 and 2 hours after the food was swallowed. Since the food protein *within the cells* at 1 hour is of similar composition to that of the food before being given to the animal, and changes only after this time, it appears that the hydra digests its food protein intracellularly.

This was further demonstrated by analyzing the food in the gut that had not been engulfed 3 hours after ingestion; this

food had the same proportions of AS and AI proteins as the original food, although, as shown in figure 42, the food within the cells had been almost completely inter-converted at this time. Thus, the food is ingested into the gastrovascular cavity, where it is partially degraded into small particles. The particles are *engulfed* by the gastrodermal cells and are subsequently *digested* and re-formed into hydra protein. Initially, the engulfed food is slowly di-

TABLE 10

Hours after Ingestion	Radioactivity (cps) in		Food Engulfed, per cent
	Hydra Tissue	Ingested Food	
1	37.2	28.2	11.6
2	83.4	72.0	53.6
3	127.3	110	53.7
5	207	65.2	79.0
9	284	34.5	89.0

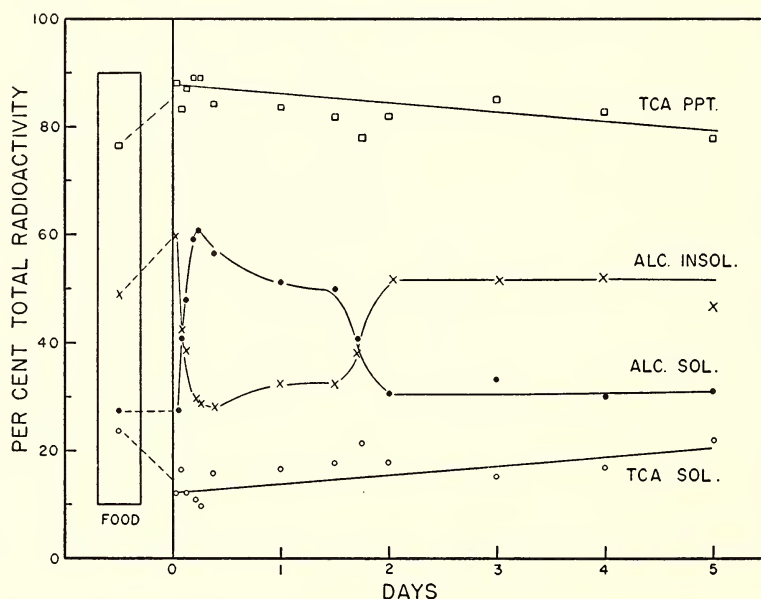


Fig. 42. Per cent distribution of S^{35} -amino acids in hydra's food (mouse lung) and also hydra's tissues which were analyzed at different periods of starvation after feeding the S^{35} -mouse lung.

gested, but after a lag it is rapidly digested. This finding suggests that the intracellular mechanisms for digestion respond adaptively to engulfed food. By measuring the total radioactivity within the hydra cells, and the total radioactivity of either the egested food or the contents of the gastrovascular cavity, the rate of protein engulfment by the hydra was determined. The results (table 10) indicate that only about 10 per cent of the ingested food is engulfed at 1 hour. By 5 hours, however, the engulfment of the particles was essentially completed and the percentage of radioactivity retained within the cells did not

differ significantly from that of the animals that had egested their wastes and were counted 9 hours after ingestion. The data presented in table 10 also indicate that protein digestion in *Hydra* is relatively efficient because the animals retain 80 to 90 per cent of the engulfed radioactive protein.

Whole animal radioautography was also used to study the digestion and redistribution of the sulfur-labeled food protein. Figure 43 (see plate 4 for figs. 43-46) is a radioautograph of an animal 1 hour after feeding. The partly degraded food tissue is seen in clumps within the gastrovascular

cavity; no radioactivity is seen in the tentacles. After 3 hours (fig. 44) the radioactivity is fairly equally distributed throughout the animal, except for the tentacles and the lower region of the tube. The absence of any radioactivity in the tentacles until 6 hours after ingestion, when traces were first seen, suggests that the endoderm of the tentacles does not function in engulfing solid particles of food. At 6 hours after ingestion the hydra had regurgitated the undigested food. A radioautograph at this time (fig. 45) revealed that most of the S^{35} was well distributed except for the lower part of the body tube, which, like the tentacles, does not seem involved in the digestion process. There does seem to be some incorporation in the basal disc, a site thought to be relatively active in synthesizing adhesive substances. There are also two nonradioactive "pockets" in the upper side of the body tube, corresponding to two testes which did not become labeled during 6 hours. This observation suggests that after the testes are formed these rapidly differentiating structures are partly independent of the rest of the animal for their nutrition.

S^{35} -labeled protein was shown, by means of the following experiment, to reach the extremities of the tentacles. The upper third of a radioactive hydra was removed, and in its place was grafted a nonradioactive hypostome of tentacles. A radioautograph of this "hybrid" (fig. 46), taken after 2 days, revealed an equal distribution of radioactivity throughout the grafted tentacles. It is probable that the mechanism of depositing much of the S^{35} -labeled material in the tentacles involves cell migration; cell migration is now definitely known to occur in the specialized cnidoblast cells whose migration is described below.

The paths of protein breakdown and synthesis in hydras starved for 1 day and then fed S^{35} -labeled mouse tissue are shown in figure 42. No significant difference is observed by comparing the TCA-soluble and TCA-precipitable fractions of

the ingested food to the same fractions of the hydra starved for as long as 5 days after feeding. But a rapid rise in the amount of an alcohol-soluble (AS) protein occurs within 6 hours; this protein remains at a high level for $1\frac{1}{2}$ days. During the next 12 hours the amount of the AS protein falls to about 30 per cent of the total radioactivity, and it remains constant at this level for at least 5 days. At the time these changes in the amount of AS protein were occurring, the alcohol-insoluble (AI) protein followed the reciprocal kinetics of degradation and synthesis. Analysis of the AS fraction demonstrated that it did not migrate on a chromatogram suspended in a butanol-formic acid solvent; thus, this fraction appears to be nonlipide and is probably entirely protein. A chromatogram of a HCl hydrolysate of the AS protein revealed the presence of all the usual *Hydra* amino acids including hydroxyproline (see below). The S^{35} was distributed between cysteine and methionine, the former being more radioactive; this distribution of radioactivity is similar to that of the mouse AS and AI proteins. On extraction with ether, some of the AS protein precipitated.

The results of this kinetic experiment indicate that once intracellular digestion starts the AI food protein is rapidly degraded and re-formed into *Hydra* AS protein. It is not yet certain, however, whether the degradation of the ingested protein proceeded to the amino acid stage before the AS protein was built up, or whether the *Hydra* AS protein is merely "chopped-up" food in the form of low-molecular-weight polypeptides or proteins. During the starvation period after feeding, the AS protein supplies amino acids or polypeptides to form *Hydra* AI protein. This "protein-to-protein" transfer may well reflect one of the mechanisms that help *Hydra* keep its body proportions relatively constant even over long periods of starvation.

The presence of hydroxyproline in the *Hydra* AS fraction is interesting because

it has recently been shown that the capsule of the *Hydra* nematocyst is an unusual member of the collagen family of proteins and contains more than 20 per cent hydroxyproline. It appears that the AS fraction may contain a precursor to the capsule.

The rate of excretion of S^{35} compounds during starvation was measured by sampling the radioactivity at 12-hour intervals in the water around hydras that had pre-

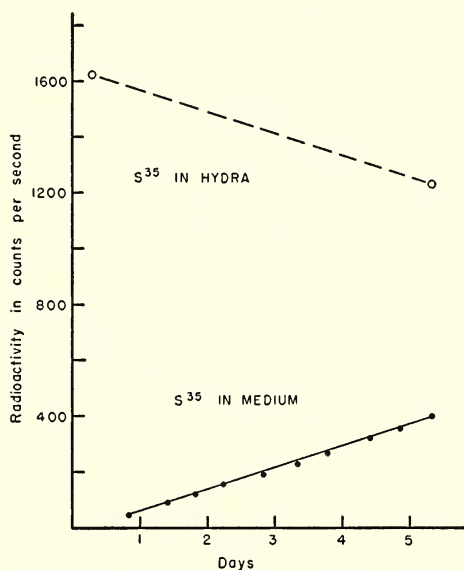


Fig. 47. Loss of S^{35} -label by a hydra on starvation, and increase of S^{35} -label in the environment over the same period.

viously regurgitated and had been washed. As demonstrated in figure 47, the hydras lose about 25 per cent of their total radioactive material at a constant rate during 5 days' starvation. If the S^{35} radioactivity can be interpreted as representative of all *Hydra* protein, these results suggest that for at least 5 days the animals are either burning up their body protein or leaking free amino acids to the outside.

The efficiency with which hydras utilized labeled tissue prompted a trial with *Planaria*, a flatworm. *Planaria*, like *Hydra*, is carnivorous, but is much more highly organized; it is triploblastic, has a more compli-

cated life cycle, and has well developed organ systems. Upon feeding planarias S^{35} -labeled mouse lung it was found that a relatively large amount of S^{35} was incorporated. Figures 48 and 49, plate 5, are radioautographs of sections of planarias killed 18 and 36 hours, respectively, after feeding. This simple experiment demonstrates the possibility of using tracer techniques for studies on lower forms and in triploblastic organisms analogous to those already made with *Hydra*. Especially intriguing problems are the conservation of protein throughout a complicated life cycle and through periods of stress, and the role of pre-existing protein during regeneration of an amputated part. In another aspect to the work on *Planaria*, egg cases were hydrolyzed and chromatographed. The chromatograms revealed the presence of a ninhydrin-positive spot at the position corresponding to α -amino-adipic acid. This amino acid has not been reported previously from animal protein sources.

Asexual Reproduction

In the earlier studies from this laboratory it was found that 25 to 30 per cent of parental P^{32} was contributed to the offspring of each asexual generation. Table 11 presents the results of a similar study using S^{35} . In the present experiments, the first bud contained about 45 per cent of the parental S^{35} and subsequent generations received 15 to 25 per cent. Figure 50, plate 5, shows that the first bud immediately receives and incorporates some of the ingested S^{35} -labeled food protein. The fourth and fifth buds, although of similar size to their earlier sibs, contain a smaller proportion of the parental S^{35} since the parent has attached buds and, therefore, more tissue. Figure 50 demonstrates that most of the bud from a S^{35} -labeled parent is evenly labeled, although the lower portion of the parent is scantily labeled. The absence of appreciable radioactivity in the gastrovascular cavity (about 0.1 per cent

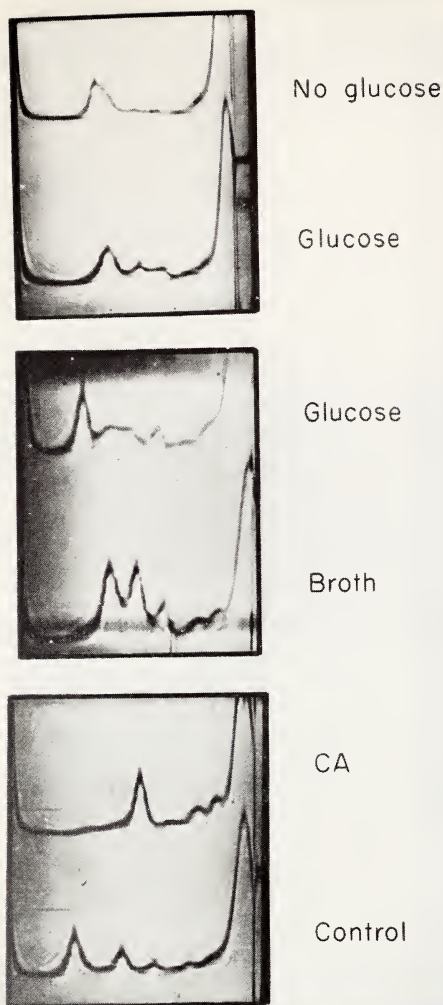


Fig. 23. Sedimentation patterns of ribonucleoproteins of *E. coli*. Each pair obtained in a single run by using a pair of analytical cells in the ultracentrifuge. *Upper*: The effect of lack of glucose; note the decrease in the amount of the smaller components. *Center*: The effect of broth; note the absence of the largest component (~ 80 S) and the increase in amount of the smaller ones. *Lower*: The effect of chloramphenicol; note the virtual absence of the largest component.

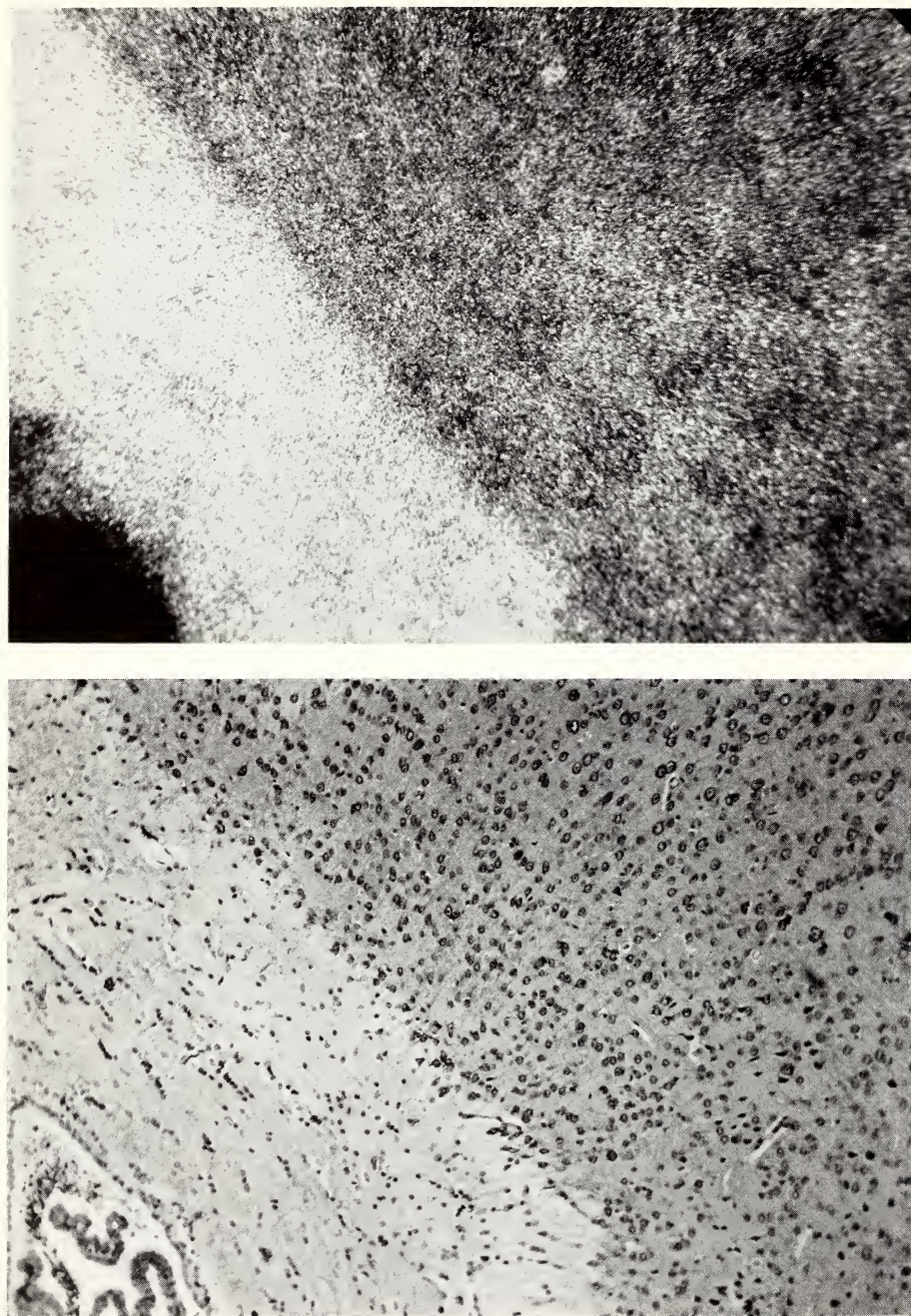


Fig. 41. Photomicrographs of thionine-stained section of cortex (*left*) and radioautograph of same section (*right*). Densely exposed region upper left, choroid plexus; intermediate lightly exposed region, nerve fibers plus glial cells; lower right, nerve cell bodies plus glial cells.



Fig. 43. Radioautograph of a hydra having S^{35} -labeled mouse lung in its gastrovascular cavity for 1 hour.

Fig. 44. Radioautograph of a hydra having S^{35} -labeled mouse lung in its gastrovascular cavity for 3 hours.

Fig. 45. Radioautograph of a hydra after it had regurgitated its undigested wastes 6 hours after ingestion of S^{35} -labeled mouse lung.

Fig. 46. Radioautograph of "hybrid" hydra consisting of S^{35} -labeled body tube grafted to an unlabeled hypostome and tentacles. The radioautograph was made 2 days after the grafting operation.



Fig. 48. Radioautograph of a longitudinal section of a "coiled" planaria that had been fed S^{35} -labeled mouse lung 18 hours before it was killed.

Fig. 49. Radioautograph of a cross section of a planaria that had been fed S^{35} -labeled mouse lung 36 hours before it was killed.

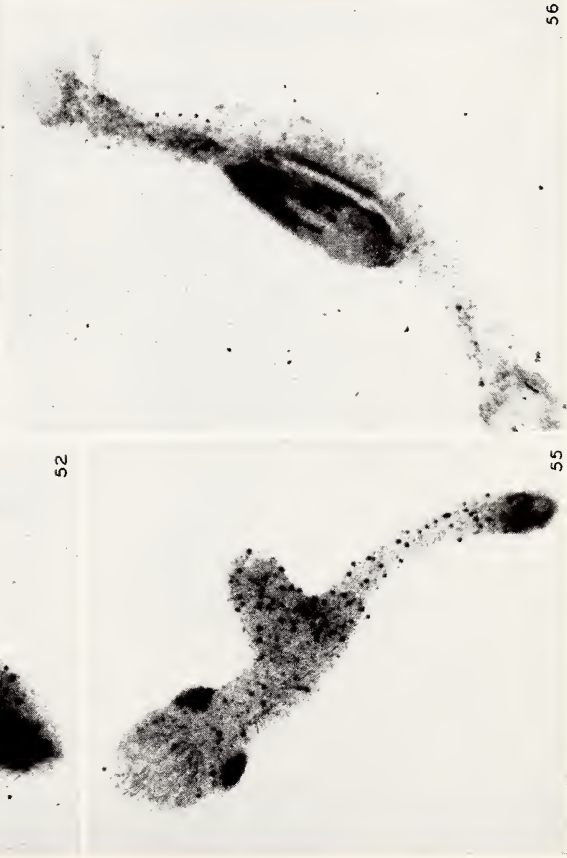
Fig. 50. Radioautograph of a bud (left) attached to its parent (right). The parent was fed S^{35} -labeled mouse lung, and subsequently fed unlabeled brine shrimp until the bud completed its development.

Fig. 51. Radioautograph of a hydra exposed to $C^{14}O_2$ for 16 hours.



53

52



56

55

54

Fig. 52. Radioautograph of a hydra exposed to C¹⁴O₂ for 16 hours, and subsequently placed in a nonradioactive environment for 48 hours.

Fig. 53. Radioautograph of tentacles and hypostome of hydras treated similarly to the hydra described for figure 50.

Fig. 54. Radioautograph of bud developed in unlabeled environment from parent exposed to C¹⁴O₂ for 16 hours.

Fig. 55. Radioautograph of a body tube of a sexually differentiating male hydra exposed to C¹⁴O₂ for 16 hours. Note two labeled testes on upper part of tube.

Fig. 56. Radioautograph of a sexually differentiating female hydra exposed to C¹⁴O₂ for 16 hours. Note heavily labeled ovary on middle part of animal.

of the total) precludes the possibility of passage of any significant protein via this cavity. It appears, therefore, that buds are derived from the more heavily labeled region in the upper part of the parent by processes that involve cellular migration as a chief element in the transport of protein from parent to offspring.

TABLE 11. Transfer of S^{35} to Buds

Bud No.	Per Cent * of Parental S^{35}			
	A	B	C	D
1	40.6	48.0	41.2	50.1
2	25.0	18.9	24.9	15.5
3	25.0	20.2	20.1	18.0
4	14.6			
5	16.2			
Remainder in parent	41.8	31.4	35.5	34.5

* This proportion was based on the amount of S^{35} in the parent just before the bud detached.

Migration of C^{14} -Labeled Cnidoblasts

The migration of the cnidoblast, which contains the developing nematocyst, is involved in the formation of the tentacles. The results below demonstrate that starved hydras can specifically incorporate C^{14} from labeled carbon dioxide into the cnidoblast. It has been possible by radioautography to demonstrate the migration of the cnidoblast from its site of origin in the body tube to its site of function. For these studies hydras in a closed vessel were exposed to $C^{14}O_2$ for various periods of time. When the required exposure had been completed, the organisms were washed, laid out on a membrane filter, killed by heat-drying, and radioautographed with Eastman NTB₃ nuclear track plates. Useful radioautographs generally required 2 to 4 weeks' exposure.

Radioautographs of animals exposed to $C^{14}O_2$ for 16 hours (fig. 51, pl. 5) revealed that most of the radioactivity was confined to small but discrete "loci" throughout the

upper two-thirds of the body tube. Few, if any, of these loci were present in the tentacles. If, on the other hand, hydras were allowed to starve in a nonradioactive environment for 48 hours after being exposed to labeled carbon dioxide, many of the loci would appear in the tentacles (fig. 52, pl. 6). This migration of the labeled loci from the body tube to the tentacles suggests that the cnidoblasts, which are making nematocysts while in the body tube, migrate to the tentacles where the nematocysts are to be used. A radioautograph of the tentacles and hypostome of a similarly treated animal demonstrates the dense concentration of radioactive nematocysts in the tentacles, and the presence of probable migratory cnidoblasts in the hypostome region while in the midst of their journey (fig. 53, pl. 6).

Experiments have been carried out in which the hydras were exposed to $C^{14}O_2$ for 16 hours during a period when a bud was just beginning on each of the animals. The bud was allowed to complete its development in a nonradioactive environment, and a radioautograph was made of that bud after it detached. As shown in figure 54, plate 6, the batteries of the bud's tentacles are strongly labeled, as are also the cnidoblasts in the body. Since the bud developed from the prelabeled parent, it apparently did not make all its own nematocysts but was given a large share of nematocysts preformed by its parent.

In experiments in which a nonradioactive hypostome with a ringlet of tentacles was grafted onto a C^{14} -labeled body tube of another animal, the radioactive cnidoblasts migrated from the body tube and were deposited along the tentacles. Thus, even tissues from another individual can be invaded by cells from the body tube.

Chemical analysis of C^{14} -labeled hydras has shown that the C^{14} is distributed among TCA-soluble, alcohol-soluble, and alcohol-insoluble fractions. Most of the radioactivity is found in glutamic and aspartic acids. This finding hints that the Krebs cycle operates in *Hydra* as it does

in other forms. In this connection it is of interest that the C^{14} label appears in those cells that are most active in synthesizing protein: cnidoblasts, which serve to re-arm the batteries of nematocysts; and testes (fig. 55, pl. 6) and ovaries (fig. 56, pl. 6), whenever these organs are being

differentiated. Thus, not only does a Krebs cycle appear to be functioning but it may serve, as in *E. coli*, to provide materials for protein synthesis. These results may have some bearing on the CO_2 induction of sexual differentiation as demonstrated by Loomis.

OPERATIONS AND STAFF

COOPERATIVE WORK OF THE DEPARTMENT

Cooperation with various institutions and organizations in this country and abroad has been continued during the year. These include the American Geophysical Union, Applied Physics Laboratory, Associated Universities, Inc., Bernard Price Institute of Geophysics (S. Africa), U. S. Coast and Geodetic Survey, Commonwealth Scientific and Industrial Research Organization (Australia), Department of Mines and Technical Surveys (Canada), Evans Signal Laboratory, Geological Survey of Canada, U. S. Geological Survey, Geophysical Institute of Huancayo (Peru), Institut Pasteur (France), International Scientific Radio Union, International Union of Geodesy and Geophysics, Johns Hopkins University, Lamont Geological Observatory, National Bureau of Standards, National Institutes of Health, National Radio Astronomy Observatory, National Research Council, National Science Foundation, Oak Ridge National Laboratory, Rocky Mountain Laboratory of the U. S. Public Health Service, Harvard, Stanford, and Yale Universities, and the Universities of Minnesota, Wisconsin, and Western Australia.

We have continued to work closely with the Geophysical Laboratory on the determination of mineral ages by means of isotope measurements.

Our cosmic-ray investigations have been aided by the cooperation of observatories at Christchurch, New Zealand; Climax, Colorado; Fredericksburg, Virginia; Godhavn, Greenland; Huancayo, Peru; and Mexico, D. F.; and by the continuation

of our contract with the Office of Naval Research covering the loan of government property.

Licenses from the Atomic Energy Commission remain in effect for the procurement of special nuclear material, and for by-product materials used in biophysical investigations.

A second contract with the Office of Naval Research, for investigations of the earth's crust, gave invaluable assistance to our Carnegie Andes Expedition through the loan of equipment.

The National Science Foundation has made two additional grants to the Institution, a total of seven such grants having been administered during the report year. One new grant provides funds for investigations and construction of photoelectric image tubes for research in astronomy; the other supports reduction of cosmic-ray ionization-chamber data in the International Geophysical Year Cosmic Ray Program.

Eight members of our staff participated in the seismic expedition to Peru, Bolivia, and Chile in the fall of 1957. Three of the members extended their travels to confer with present and potential colleagues in Argentina, Brazil, Ecuador, and Venezuela. Another staff member spent nearly two months in South America in connection with the International Geophysical Year project concerned with the height of the equatorial electrojet in the ionosphere. Several staff members have continued to serve on committees and panels for the Biophysical Society, Federal Civil Defense Administration, International Geophysical

Year, International Scientific Radio Union, and the National Academy of Sciences.

ADMINISTRATION AND OPERATION

Publication of the *Journal of Geophysical Research* has continued during the report year, with partial subsidy from the

Institution. Preliminary plans have been made for the American Geophysical Union to take over publication of the *Journal* in 1959.

Rental of more than 100 acres for radio astronomy activities has been continued at the River Road site.

BIBLIOGRAPHY

- Abelson, P. H. See Major Publications below.
- Anderson, C. E. See Heydenburg, N. P.
- Balsley, J. R. See Graham, J. W.
- Barloutaud, R., T. Grjebine, P. Lehmann, A. Lévêque, J. Quidort, and G. M. Temmer. Recherche de l'excitation coulombienne du deuxième niveau de ^{194}Pt . *Compt. rend. acad. sci. Paris*, 244, 187-190 (1957).
- Baum, W. A., W. K. Ford, Jr., and J. S. Hall. Recent astronomical tests of thin-film image converters. (Abstract.) *Astron. J.*, 63, 47-48 (1958).
- Bolton, E. T., and J. Leahy. Association of metabolic pool material with structural components of *Escherichia coli*. (Abstract.) *Natl. Biophys. Conf.*, Columbus, Ohio, pp. 11-12 (1957).
- Bolton, E. T., and H. G. Mandel. The effects of 6-mercaptapurine on biosynthesis in *Escherichia coli*. *J. Biol. Chem.*, 227, 833-844 (1957).
- Bolton, E. T. See also Cowie, D. B.; Hoyer, B. H.; and Major Publications below.
- Britten, R. J., and F. T. McClure. Osmotic effects in *Escherichia coli*. (Abstract.) *Natl. Biophys. Conf.*, Columbus, Ohio, p. 14 (1957).
- Britten, R. J. See also Cowie, D. B., and Major Publications below.
- Buddington, A. F. See Graham, J. W.
- Burke, B. F. Confusion effects in surveys of radio sources. (Abstract.) *URSI Program of Joint Meeting*, National Bureau of Standards, Apr. 23-26, 1958, p. 16.
- Burke, B. F., and J. W. Firor. Limiting accuracy of open-wire transmission lines in positional radio astronomy. (Abstract.) *URSI Program of Joint Meeting*, National Bureau of Standards, Apr. 23-26, 1958, p. 16.
- Burke, B. F., and K. L. Franklin. Jupiter as a radio source. I.A.U. Symposium 4, *Radio Astronomy* (Manchester meeting, 1955), edited by H. C. van de Hulst, Cambridge University Press, pp. 394-396 (1957).
- Burke, B. F., and K. L. Franklin. Observations of discrete sources with the 22 Mc/s Mills Cross. I.A.U. Symposium 4, *Radio Astronomy* (Manchester meeting, 1955), edited by
- H. C. van de Hulst, Cambridge University Press, pp. 151-153 (1957).
- Cohen, G. N. See Cowie, D. B.
- Cowie, D. B., and E. T. Bolton. The use of metabolic pools of purine compounds for nucleic acid synthesis in yeast. *Biochim. et Biophys. Acta*, 25, 292-298 (1957).
- Cowie, D. B., and R. J. Britten. Kinetic studies of metabolic pools in microorganisms. (Abstract.) *Natl. Biophys. Conf.*, Columbus, Ohio, p. 22 (1957).
- Cowie, D. B., and G. N. Cohen. Biosynthesis by *Escherichia coli* of active altered proteins containing selenium instead of sulfur. *Biochim. et Biophys. Acta*, 26, 252-261 (1957).
- Cowie, D. B. See also Lederberg, J., and Major Publications below.
- Davis, G. L. See Tilton, G. R.
- Erickson, W. C. A mechanism of non-thermal radio-noise origin. *Astrophys. J.*, 126, 480-492 (1957).
- Firor, J. W. A radio telescope. *QST*, 41, 32-36 (1957).
- Firor, J. W. Brightness distribution of the sun at 1.45 metres. I.A.U. Symposium 4, *Radio Astronomy* (Manchester meeting, 1955), edited by H. C. van de Hulst, Cambridge University Press, pp. 294-297 (1957).
- Firor, J. W. The slowly varying component of solar radiation at 340 Mc. (Abstract.) *URSI Program of Joint Meeting*, National Bureau of Standards, Apr. 23-26, 1958, p. 15.
- Firor, J. W. See also Burke, B. F.
- Forbush, S. E. Large increase of cosmic-ray intensity following solar flare on February 23, 1956. (Letters to Editor.) *J. Geophys. Research*, 61, 169-170 (1957).
- Forbush, S. E. See also Major Publications below.
- Ford, W. K., Jr. See Baum, W. A.
- Frankel, S., P. G. Hansen, O. Nathan, and G. M. Temmer. Detection of Ga^{66} positron polarization by the annihilation-in-flight rate in polarized matter. *Phys. Rev.*, 108, 1099-1101 (1957).
- Franklin, K. L. See Burke, B. F.
- Graham, J. W. The role of magnetostriction in rock magnetism. *Phil. Mag. Supplement*, 6, 362 (1957).

- Graham, J. W., A. F. Buddington, and J. R. Balsley. Stress-induced magnetizations of some rocks with analyzed magnetic minerals. *J. Geophys. Research*, 62, 465-474 (1957).
- Grjebine, T. See Barloutaud, R.
- Hall, J. S. See Baum, W. A.
- Hansen, P. G. See Frankel, S.
- Heydenburg, N. P., and G. F. Pieper. Coulomb excitation of rotational levels in dysprosium. *Phys. Rev.*, 107, 1297-1299 (1957).
- Heydenburg, N. P., G. F. Pieper, and C. E. Anderson. Coulomb excitation of krypton. *Phys. Rev.*, 108, 106-107 (1957).
- Heydenburg, N. P. See also Pieper, G. F.; and Temmer, G. M.
- Hoyer, B. H., E. T. Bolton, R. A. Ormsbee, G. LeBouvier, D. B. Ritter, and C. L. Larson. Mammalian viruses and rickettsiae. *Science*, 127, 859-863 (1958).
- Lange, I. See Major Publications below.
- Larson, C. L. See Hoyer, B. H.
- Leahy, J. See Bolton, E. T.
- LeBouvier, G. See Hoyer, B. H.
- Lederberg, J., and D. B. Cowie. Moondust. *Science*, 127, 1473-1475 (1958).
- Lehmann, P. See Barloutaud, R.
- Lévêque, A. See Barloutaud, R.
- Mandel, H. G. See Bolton, E. T.
- McClure, F. T. See Britten, R. J.; and Roberts, R. B.
- Nathan, O. See Frankel, S.
- Ormsbee, R. A. See Hoyer, B. H.
- Pieper, G. F., and N. P. Heydenburg. Coulomb excitation of iron-57. *Phys. Rev.*, 107, 1300-1302 (1957).
- Pieper, G. F. See also Heydenburg, N. P.
- Quidort, J. See Barloutaud, R.
- Ritter, D. B. See Hoyer, B. H.
- Roberts, R. B., and F. T. McClure. Models of *Escherichia coli* relating structure and function. (Abstract.) *Natl. Biophys. Conf.*, Columbus, Ohio, p. 59 (1957).
- Roberts, R. B. See also Major Publications below.
- Scott, W. E. List of recent publications. *J. Geophys. Research*, 62, 500-507, 650-658 (1957); 63, 257-264, 429-436 (1958).
- Tatel, H. E. 21-cm meridian plane surveys. I.A.U. Symposium 4, *Radio Astronomy* (Manchester meeting, 1955), edited by H. C. van de Hulst, Cambridge University Press, pp. 67-70 (1957).
- Tatel, H. E., and M. A. Tuve. Carnegie seismic expedition to the Andes, 1957. (Abstract.) *Trans. Am. Geophys. Union*, 39, 580 (1958).
- Tatel, H. E., and M. A. Tuve. Seismic observations at one kilometer depth. *Contributions in Geophysics: Honor of Beno Gutenberg*, Pergamon Press, London, pp. 152-157 (1958).
- Temmer, G. M. Science in the USSR. "Nuclear Physics," *Science*, 126, 1097 (1957).
- Temmer, G. M., and N. P. Heydenburg. Low-lying excited states of Na²². (Abstract.) *Bull. Am. Phys. Soc.*, ser. II, 3, 200 (1958).
- Temmer, G. M. See also Barloutaud, R.; and Frankel, S.
- Tilton, G. R., G. L. Davis, and G. W. Wetherill. The age of the Baltimore gneiss. (Abstract.) *Program Am. Geophys. Union*, May 5-8, 1958, p. 42.
- Tuve, M. A. See Tatel, H. E.
- Vestine, E. H. Geomagnetic field. *The Earth and Its Atmosphere*, edited by D. R. Bates, Basic Books, Inc., New York, pp. 89-96 (1957).
- Wells, H. W. Flux measurements of Cassiopeia A and Cygnus A between 18.5 Mc and 107 Mc. *Proc. IRE*, 46, 205-208 (1957).
- Wells, H. W. International cooperation in radio research. *Dept. State Bull.*, 37, 897-899 (1957).
- Wells, H. W. Large-scale movements of the layers. *J. Atmospheric Terrest. Phys.* (Special Supplement), Proc. Polar Atmos. Symposium Part II, Ionosphere Section, Oslo, pp. 33-40 (1957).
- Wells, H. W. Preliminary observations of point source at 12.5 and 15.5 Mc/s. I.A.U. Symposium 4, *Radio Astronomy* (Manchester meeting, 1955), edited by H. C. van de Hulst, Cambridge University Press, pp. 148-150 (1957).
- Wells, H. W. Unusual propagation at 40 Mc from the USSR satellite. *Proc. IRE*, 46, 610 (1958).
- Wetherill, G. W. Radioactivity of potassium and geologic time. *Science*, 126, 545-549 (1957).
- Wetherill, G. W. See also Tilton, G. R.

MAJOR PUBLICATIONS

- Cosmic-Ray Results*. Huancayo, Peru, January 1946-December 1955; Cheltenham, Maryland, March 1936-December 1955; Christchurch, New Zealand, January 1947-December 1955; Godhavn, Greenland, January 1947-December 1950. By I. Lange and S. E. Forbush. *Carnegie Inst. Wash. Publ.* 175, XX. Quarto, v + 224 pp., 431 tables. August 1957.
- Studies of Biosynthesis in Escherichia coli*. By R. B. Roberts, P. H. Abelson, D. B. Cowie, E. T. Bolton, and R. J. Britten. *Carnegie Inst. Wash. Publ.* 607. Octavo, xiv + 521 pp., 114 figs., second printing with brief addenda. September 1957.

PERSONNEL

Director

M. A. Tuve

Staff Members

L. T. Aldrich	J. W. Graham (resigned February 8, 1958)
E. T. Bolton	N. P. Heydenburg
R. J. Britten	R. B. Roberts
B. F. Burke	H. E. Tatel (deceased November 15, 1957)
D. B. Cowie	G. M. Temmer
J. W. Firor	H. W. Wells
S. E. Forbush	G. W. Wetherill
W. K. Ford, Jr. (from September 3, 1957)	

Section Chairmen

Atmosphere: H. W. Wells	Nuclear Physics: N. P. Heydenburg
Biophysics: R. B. Roberts	Theoretical Geophysics: S. E. Forbush
Earth's Crust: H. E. Tatel to November 15, 1957; L. T. Aldrich from December 12, 1957	

Fellows and Associates

G. N. Cohen, Institut Pasteur, Paris, France (part time)	tion, Sydney, Australia (from August 1957 to May 1958)
D. W. Dewhirst, University of Cambridge, Cambridge, England (entire report year)	G. F. Pieper, Yale University (to August 1957)
W. C. Erickson (to August 1957)	I. Z. Roberts, Trinity College (part time)
H. L. Helfer (to August 1957)	H. de Robichon-Szulmajster, National Institutes of Health (part time)
J. J. Leahy (to August 1957)	J. O. Thomas, University of Cambridge, Cambridge, England (April 1958)
H. M. Lenhoff (from February 1, 1958)	H. Weaver, University of California (to August 1957)
D. H. Lindsley, Johns Hopkins University (part time)	
J. L. Pawsey, Australian Commonwealth Scientific and Industrial Research Organiza-	

Visiting Investigators

Carl E. Anderson, Yale University (September 1957)	D. R. Marshall, University of Chicago (October 1957)
J. A. Clegg, United Kingdom (September 1957)	F. T. McClure, Applied Physics Laboratory (part time)
R. D. Davies, Jodrell Bank Experimental Station, Cheshire, England (July 1957)	Catharine Mouquin, Trinity College (part time from February 1958)
L. B. and Mrs. J. B. Flexner, University of Pennsylvania (part time)	L. O. Nicolaysen, Bernard Price Institute of Geophysics, Johannesburg, S. Africa (April, May 1958)
S. Gorodetsky, Institute of Nuclear Research, Strasbourg, France (March 1958)	E. C. Pollard, Yale University (September 1957)
B. H. Hoyer, Rocky Mountain Laboratory (April 1958)	P. German Saa, S.J., Universidad del Norte, Antofagasta, Chile (January 1958)
C. Lomnitz, University of Chile, Santiago, Chile (June 1958)	G. J. Wasserburg, California Institute of Technology (April 1958)
H. G. Mandel, George Washington University (part time)	

Research Assistants

J. B. Doak
E. T. Ecklund

C. A. Little, Jr.
W. E. Scott

Laboratory Assistants

Miss R. E. Bresnahan
S. J. Buynitzky
Miss E. F. French (to August 23, 1957)
P. A. Johnson

Miss B. D. North
R. W. Rasmussen (from April 1, 1958)
R. W. Reuschlein (to February 28, 1958)

Office

Chief, Administrative and Operating Section:
M. B. Smith (retired December 31, 1957)
Chief, Fiscal Section: Miss H. E. Russell
(from January 1, 1958); Accountant (to
December 31, 1957)

Director's Secretary and Office Manager:
W. N. Dove
Librarian: Mrs. L. J. Prothro
Stenographers: Mrs. C. Ator; Mrs. A. L.
Hill (from May 14, 1958); Mrs. C. Wind-
muller (to May 23, 1958)

Shop

Chief of Subsection—Main Shop and Main-
tenance: W. F. Steiner
Senior Instrument Makers: B. J. Haase;
L. A. Horton (retired June 30, 1958);
J. G. Lorz

Machinist: F. J. Caherty
Machinist-Instrument Maker: M. Seemann
(from June 16, 1958)

Buildings and Grounds

Foreman: C. Balsam
Caretaker: E. Quade

Assistant Caretakers: C. R. Forshier; S.
Gawrys; S. Swantkowski

Part-Time and Temporary Employees

Mrs. L. Beach
R. W. Bluehdorn
M. S. Halversen
R. C. Kile
F. R. Norcross
N. E. Peppell

R. L. Peppell
K. W. Schwarz
Mrs. M. T. Sheahan
C. F. Stroebel
A. R. Turpin
W. W. Wyatt

GEOPHYSICAL LABORATORY

Washington, District of Columbia

PHILIP H. ABELSON, *Director*

CONTENTS

	page		page
<i>Introduction</i>	169	The quaternary system $\text{Na}_2\text{O-MgO-Al}_2\text{O}_3\text{-SiO}_2$	210
<i>Experimentation at High Pressure</i>	170	Systems with rock-forming olivines, pyroxenes, and feldspars	212
Development of high-pressure apparatus	170	<i>Paleobiochemistry</i>	214
Single-stage apparatus	170	<i>Ore Minerals</i>	214
Two-stage apparatus	172	The Cu-S system	215
Melting of diopside under high pressure	173	The Fe-S system	218
Melting points of alkali halides at high pressure	174	The solidus in the system Cu-Fe-S between 400° and 800° C.	222
<i>The Age of Rocks and Minerals</i>	176	The Fe-Zn-S system	227
Appalachian ages	177	The Fe-As-S system	229
Other ages	179	The $\text{CoAs}_2\text{-NiAs}_2\text{-FeAs}_2\text{-As}$ system ..	232
North American ages	181	Sulfide-water systems	234
Acknowledgments	181	Ore solutions	234
<i>Mineral Assemblages in the Green River Formation</i>	181	<i>Diffraction Effects of Short-Range Ordering in Layered Sequences</i>	240
<i>Geochemistry of Artificial Isotopes</i>	184	The distribution of run lengths	240
The beneficiation of soils contaminated with strontium 90; beneficial effects of potassium	184	Generation of diffraction masks of layered sequences	241
Rain fallout	186	The optical diffractometer	242
Tritium hydrology	189	The calculation of intensity profiles ..	242
<i>Experimental Petrology</i>	189	<i>Crystallography</i>	243
Effect of water on the melting of silicates	189	Neutron diffraction studies	243
Iron-rich chlorites	191	Magnetic structure of chalcopyrite ..	243
Biotites	192	Symmetry of magnetic structures ..	244
Phase relations of hydrous silicates with intermediate Mg/Fe ratios ..	193	Sulfides	246
The phlogopite-annite join	194	High-temperature chalcopyrite	246
Ferrous-ferric biotites	195	Arsenopyrite	246
Cordierite- H_2O system	195	Bornite	248
Polymorphism	196	Silicates	249
Composition	196	Pyroxenes	249
Lower stability limits	196	Synthetic mica of type 3M	252
Anhydrous alkali-free cordierites	197	<i>Miscellaneous Administration</i>	253
Alkali amphiboles	199	Petrologists' Club	253
Magnesioriebeckite	199	Seminars	253
Glaucophane	202	Johns Hopkins University and Geological Laboratory graduate seminars on "Researches in geochemistry"	254
Riebeckite	204	Symposium on C^{14} dating, Pleistocene stratigraphy, and archaeological chronology	255
Composition of a pseudoleucite from the Bearpaw Mountains, Montana	204	Lectures	255
Feldspar investigations	206	<i>Summary of Published Work</i>	256
Spinel	209	<i>Bibliography</i>	259
Magnetite-hercynite relations	209	<i>Personnel</i>	259
Chester emery deposits	210		

INTRODUCTION

Geochemical and geophysical research are currently making exciting progress. Unfortunately, publications on these topics are widely scattered and are often effectively lost to most graduate students and professional earth scientists. The Geophysical Laboratory has one successful partial solution to this problem. Requests for our annual reports, which contain most of our results, come from individuals and libraries all over the world. More than 1500 copies are sent out each year, over a third of them to foreign countries.

During the past year this Laboratory made an additional effort in the collection and dissemination of scientific information related to our area of activity. A series of weekly seminars and discussions on the subject "Researches in Geochemistry" was held both at the Johns Hopkins University in Baltimore and at this Laboratory during the academic year 1957-1958. Some of the leading geochemists of the country participated. The manuscripts they submitted, covering their remarks, have been edited at the Laboratory and will be published early in 1959 by John Wiley & Sons, Inc., New York. The volume, containing 23 chapters, should be helpful in many ways to students and to more mature investigators. It provides reviews of progress in many interesting areas by men currently active in research. It describes much new and previously unpublished work, and includes many excellent bibliographies. A list of speakers and titles is given in a later section of the present report.

Our diversified experimental program made substantial progress in many areas. Boyd, S. Clark, and England have been designing, constructing, and testing new equipment for geochemical and geophysical studies at high pressures. One new device is capable of maintaining 50,000 atmospheres at 1700° C simultaneously.

Tilton and Davis, together with col-

leagues at the Department of Terrestrial Magnetism, have measured the ages of many rocks and minerals, including a group from the Appalachian chain. The pre-existing gneisses have been dated at 1100 million years (zircons) and the orogeny at about 300 million (micas).

Eugster and Milton (U. S. Geological Survey) have applied phase-equilibria chemical considerations to explain some of the bizarre mineral assemblages of the Green River shale.

Libby has carried on a series of researches on geochemistry of fission products, particularly Sr⁹⁰.

Yoder has expanded his studies of the effect of water on the melting relations of rock-forming silicates, and summarizes all the available examples.

Eugster, Wones, and Turnock have studied pressure-temperature stability characteristics of a series of hydrous, iron-bearing micas and chlorites.

Additional investigations in experimental petrology include a study of cordierites by Yoder, Schairer, and Schreyer; of amphiboles by Ernst; of pyroxenes by Schairer and Morimoto; and of spinels by Wones and Turnock.

The ore minerals have been the subject of intensive research by a group consisting of Kullerud, Arnold, Barnes, L. Clark, and Roseboom. More than a score of systems are under study, and many new data which will ultimately be applicable to geothermometry of ore deposits have been obtained.

Chayes has continued his experimental investigations of order-disorder diffraction effects both with optical analogs and by high-speed calculations.

Crystallographic studies have been pursued intensively by Donnay and Morimoto, Donnay being particularly active in studies of sulfides. A collaborative study of chalcopyrite by neutron diffraction procedures

has provided many new data about the magnetic structure of this mineral, and indicates that the substance is best repre-

sented by the formula $\text{Cu}^+\text{Fe}^{+++}\text{S}_2$. This and other researches are described in more detail in the report that follows.

EXPERIMENTATION AT HIGH PRESSURE

DEVELOPMENT OF HIGH-PRESSURE APPARATUS

F. R. Boyd and J. L. England

Research at high pressures this year has been primarily devoted to the development of equipment, although some preliminary phase-equilibria data have been obtained and are presented below. Equipment development is necessary to reach the pressures and temperatures required to find answers to many problems relating to the nature of the upper portion of the earth's mantle. With the "squeezer" apparatus, previously described, we have been able to reach pressures approaching 100,000 atm. Such pressures, however, may be attained with this device only at relatively low temperatures (300° to 400° C). Moreover, our work with the transition quartz \rightleftharpoons coesite in the squeezer revealed pressure gradients up to 30 per cent of the total pressure. Such gradients are probably not present in all systems investigated with this apparatus, but they lead to an uncertainty that is large in relation to the applications to which the data may be put. It has been our aim to develop equipment which would extend the temperature and pressure range beyond that attainable with the squeezer, and which would be a pressure system hydrostatic within small, known limits.

We have been working with two types of apparatus. Our single-stage apparatus is now on a fully operational basis at pressures up to 50,000 bars and temperatures up to 1750° C. The limitation on temperature is a measurement limit; a platinum/platinum-10 per cent rhodium thermocouple melts at a temperature slightly above 1750° C. We are experimenting with a tungsten-iridium thermocouple in an effort to extend our measurable range above 1750° C.

At a pressure of about 50,000 bars the limit of strength of the piston in our single-stage apparatus is reached. We have developed a method of supporting this piston to reach pressures above 50,000 bars. Apparatus with a supported piston, described below as our two-stage apparatus, has been tested to a pressure of 65,000 bars at a temperature of 1100° C.

We have begun to accumulate data on a variety of systems. A particular interest is in obtaining data on the change in melting point of various silicates with pressure. Such data are important because they place a limit on the geothermal gradient within the mantle. Preliminary data on the melting of diopside are presented below. In addition to the work on melting curves, we are looking into new solid-state reactions and checking diagrams previously worked out with the squeezer.

Single-Stage Apparatus

Our single-stage apparatus is shown in figure 1. In developing this design we have benefited from the experience of Tracy Hall and Loring Coes, who have used somewhat similar apparatus. The sample and furnace assembly are contained in a carbide pressure vessel supported by a steel ring. Pressure is applied by driving a carbide piston into the pressure vessel. Power for the furnace is supplied through a stainless-steel plug insulated by a ceramic ring. Up to four thermocouple leads in a ceramic tube may be introduced into the pressure chamber through a hole in the power lead. The sample, which consists of about 25 mg of powder in a platinum capsule, is located in the center of a graphite furnace $1\frac{1}{8}$ inches in length.

Tests have shown that the pressure gradient within the furnace and run assembly is less than 5 per cent. By a calibration pro-

cedure described below, the pressure on the run may be measured with an uncertainty of less than ± 3 per cent. Despite the small size of the furnace, the temperature gradient is small. At 500°C we have measured a temperature difference of 5° between two thermocouples, one placed in the position of the run and one placed $\frac{1}{8}$ inch below.

these vessels has failed explosively. The steel ring is machined from AISI 4340, hardened to Rockwell C45, and stretched on a mandrel. Stretching a ring expands its bore 1 per cent and increases its yield point by a factor of about 2. The carbide core with an outside diameter of 2 inches is fitted to the ring with an interference of 0.018 inch. Tapering the ring and core

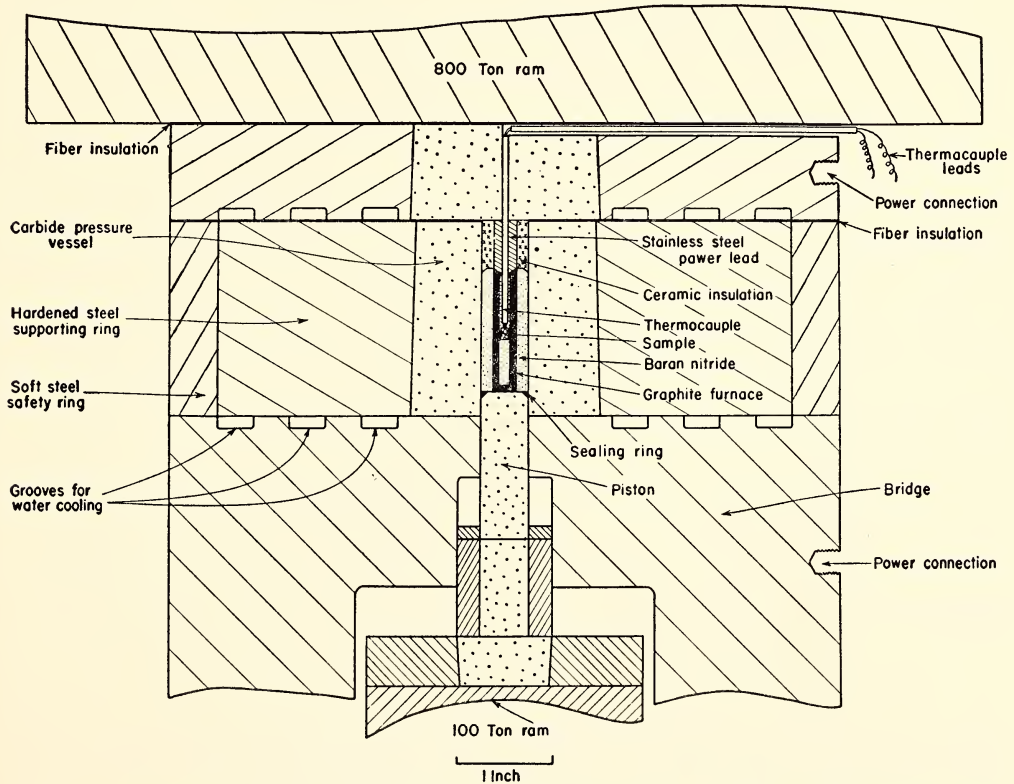


Fig. 1. Apparatus for phase-equilibrium investigations in the range 10 to 50 kilobars and at temperatures up to 1750°C . Steel parts are ruled, carbide parts stippled.

Our single-stage apparatus has provision both for end-loading the carbide pressure vessel and for water cooling. End-loading the pressure vessel extends its life. Water cooling makes it possible to run at a steady state at 1750°C . Without water cooling, the steel supporting ring expands away from the carbide and causes its rapid deterioration.

Our pressure vessels have a life of more than 20 runs at high temperature. None of

with a total angle of 1° enables them to be readily pushed together in a hydraulic press. The carbide core gradually cracks up with use. When no longer usable, the core can be pushed out and the ring fitted with new carbide.

Pressure calibration in our apparatus is achieved by measuring the transitions in bismuth. These lie at 25 and 27 kilobars at room temperature, and they have been accurately determined in special apparatus

by Bridgman. We have used them to calibrate our apparatus, and to study the effectiveness of various solid pressure media. The bismuth transitions are readily detected by measuring the change in electrical resistance in bismuth with pressure. The bismuth in the form of a strip of foil $\frac{1}{4}$ inch long and about 0.002 inch thick is

range 5 to 7 per cent. Jacketing the specimens of solid pressure media in lead foil greatly reduces the friction. Unjacketed teflon gives a friction of 16 per cent; with lead foil the friction is reduced to 5 per cent. The hysteresis on these friction runs is normally about three times the friction measured on raising the pressure.

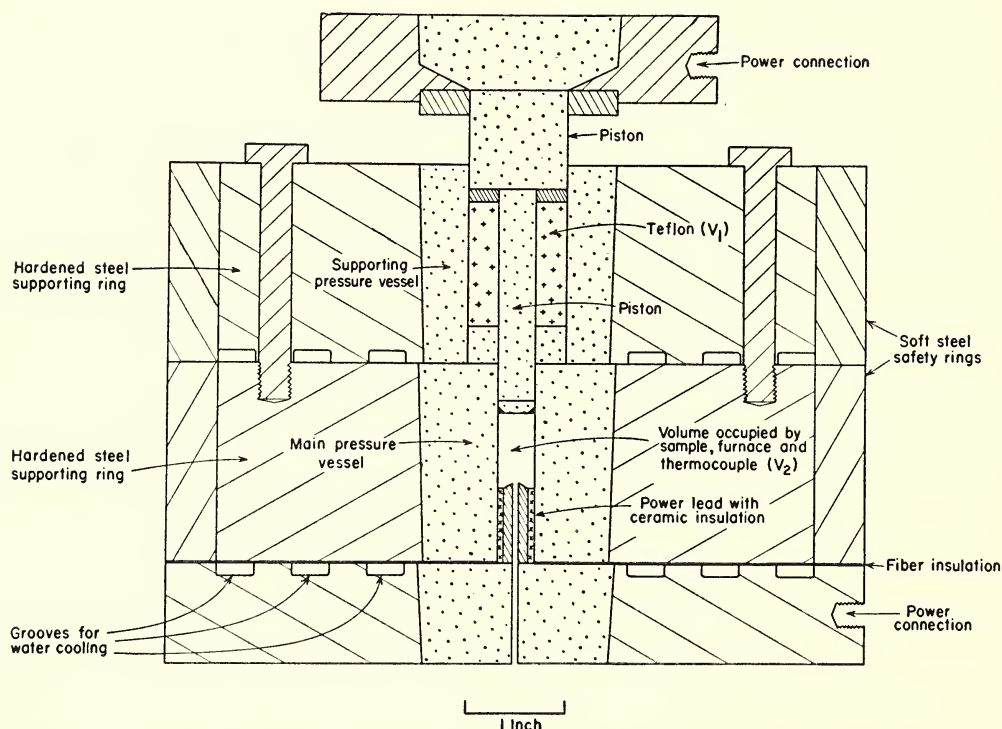


Fig. 2. Apparatus for use in the pressure range over 50 kilobars. Steel parts are ruled, carbide parts stippled. The furnace and run assembly placed in V_2 is similar to that illustrated in figure 1.

mounted between two cylinders of a solid pressure medium. Gold leads are used for electrical connections. The bismuth foil can be mounted at any level in a column of solid pressure medium, and hence pressure gradients can readily be studied. The solid pressure media we have studied are boron nitride, talc, lavastone (pyrophyllite), teflon, and Solenhofen limestone. With boron nitride jacketed in lead foil we have measured friction as low as 2 per cent at 25 kilobars. The other pressure media, jacketed in lead, yield values in the

Two-Stage Apparatus

Figure 2 illustrates the present stage of evolution of our two-stage apparatus. The main pressure vessel is constructed and supported in the same manner as in our single-stage apparatus, but a second stage, which supports the piston, is added. There are two volumes, V_1 and V_2 , under pressure. V_2 , the high-pressure volume, houses the sample and furnace assembly; V_1 contains a material (teflon in most of our runs) which supports the piston. The relations between the volumes, the pressures

P_1 and P_2 in these volumes, the compressibilities B_1 and B_2 of the materials in V_1 and V_2 , and the areas A_1 and A_2 over which the piston bears are given by the equation

$$\frac{P_1}{P_2} = \frac{(V_2/A_2)B_2}{(V_1/A_1)B_1}$$

If the volumes are cylindrical, as in the design in figure 2, the equation may be simplified to

$$P_1/P_2 = L_2 B_2 / L_1 B_1$$

where L_1 and L_2 are the lengths of the volumes. By adjusting the lengths of the volumes and selecting pressure media with different compressibilities, the ratio of run pressure to supporting pressure can be varied. We are currently working with a ratio of $P_2:P_1$ of about 3:1.

In principle, the pressure distribution between the two volumes can be calculated from a knowledge of the total load on the piston, the friction, the lengths of V_1 and V_2 , and the relative compressibilities of the materials in V_1 and V_2 . In practice, V_2 will ordinarily contain a complex furnace, thermocouple, and run assembly with initial voids, and the compressibility of the assembly is not known well enough to permit adequate computation of P_2 .

We have, consequently, developed a method of calibration that permits us to measure P_2 with an accuracy of about ± 5 per cent. We calibrate by putting known loads on V_1 and measuring the advance of the piston as a function of load. During calibration the run assembly in V_2 is replaced by a piston driven by a separate hydraulic press the load on which is known. With a run assembly in V_2 , measurement of piston advance yields at once the load on V_1 , and by difference with the total load we have the load on V_2 . This method has the particular advantage that the friction in V_1 need not be independently estimated, since it is automatically taken into account in the calibration. The friction in V_2 can be estimated by making

a run on a known transition. We have found the friction in V_2 with lavastone as the pressure medium to be 6 per cent at the bismuth point, in agreement with our data from single-stage apparatus.

Our range with the two-stage apparatus is currently about 65,000 at temperatures above 1000° C; we believe that minor improvements will extend the range considerably beyond 65,000 bars.

MELTING OF DIOPSIDE UNDER HIGH PRESSURE

F. R. Boyd and J. L. England

Accurate estimate of the variation of temperature with depth in the earth's mantle is of major importance to the solution of many geophysical problems. Seismic data tell us that rocks in the earth's mantle behave as predominantly crystalline solids. Knowledge of the effect of pressure on the melting points of certain refractory silicates that we can infer to be constituents of the mantle permit us to place an upper limit on the geothermal gradient.

Preliminary data on the effect of pressure on the melting of diopside are presented in figure 3. These data were obtained in our single-stage apparatus. Yoder (1952) found that the melting point of diopside increases with pressure at the rate of 13.0° C per 1000 bars in the range 1 to 5000 bars. Present data extend the melting curve of diopside to 32,000 bars and 1740° C. The slope of the melting curve decreases with pressure; in the range 20,000 to 30,000 bars the average slope is 10.3° per 1000 bars. A smooth curve can be drawn through our points and the data obtained by Yoder.

Diopside liquid cannot be quenched to a glass above about 11,000 bars, but we have been able to use a pronounced textural change in the run to locate the melting curve at higher pressures. The curve presented has been corrected for friction in our apparatus but not for the effect of pressure on the emf of the thermocouple. Present data indicate that this effect is small.

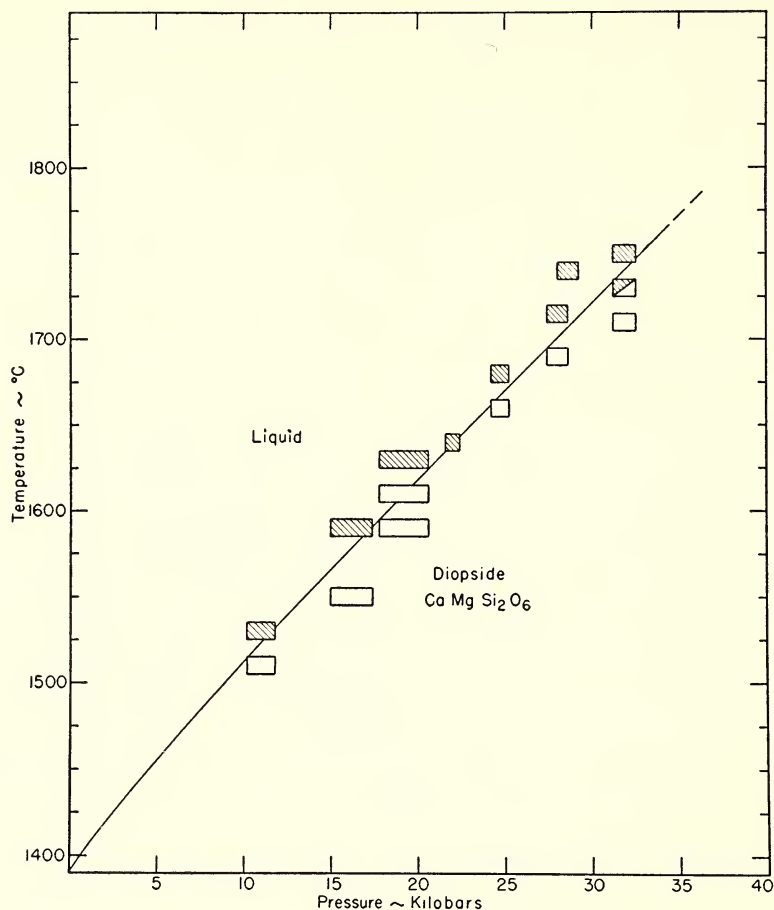


Fig. 3. Preliminary curve for the melting of diopside under pressure.

MELTING POINTS OF ALKALI HALIDES AT HIGH PRESSURE

S. P. Clark, Jr.

The effect of pressure on melting temperatures is of considerable interest in the physical theory of liquids. It is also of immediate geophysical importance. Pressure increases with depth in the earth, and present data indicate that the temperature does likewise. Since large volumes of lava have been poured out at the surface of the earth in every period of geologic time, partial fusion of the material at depth in the earth must commonly take place.

Previous work on melting relations at high pressure has largely been confined to metals, organic compounds, and van der

Waals solids such as He, H₂, and N₂. Simon found that many experimentally determined melting curves could be represented by the equation

$$P/P_0 = (T/T_0)^c - 1$$

where T_0 is the melting temperature at zero pressure (or at the solid-liquid-vapor triple point), and P_0 and c are adjustable constants.

Subsequent theoretical investigation has shown that this equation, originally considered to be empirical, can be derived from the Lindemann melting criterion. The derivation also leads to theoretical values of P_0 and c , which prove to be related to parameters appearing in the Mie-Grüneisen equation of state.

Although the applicability of the Simon equation can be tested on material of any kind, the relation between P_0 and c as determined from melting curves, and their theoretical values, can be examined only if the theoretical values can be calculated. The calculation assumes that the substances are fairly accurately represented by the simple models of solids developed by Debye, Gruneisen, and Born, and it further requires reasonably complete thermo-

shown in figures 4 and 5. The breaks in slope in the curves for KCl, RbCl, and CsCl result from the intersection of the melting curves with curves representing the appearance of solid polymorphs. The high-pressure form of CsCl on the melting curve is the same as the form stable at room temperature and atmospheric pressure. The other polymorphic inversions are to true high-pressure phases which had previously been discovered at low tempera-

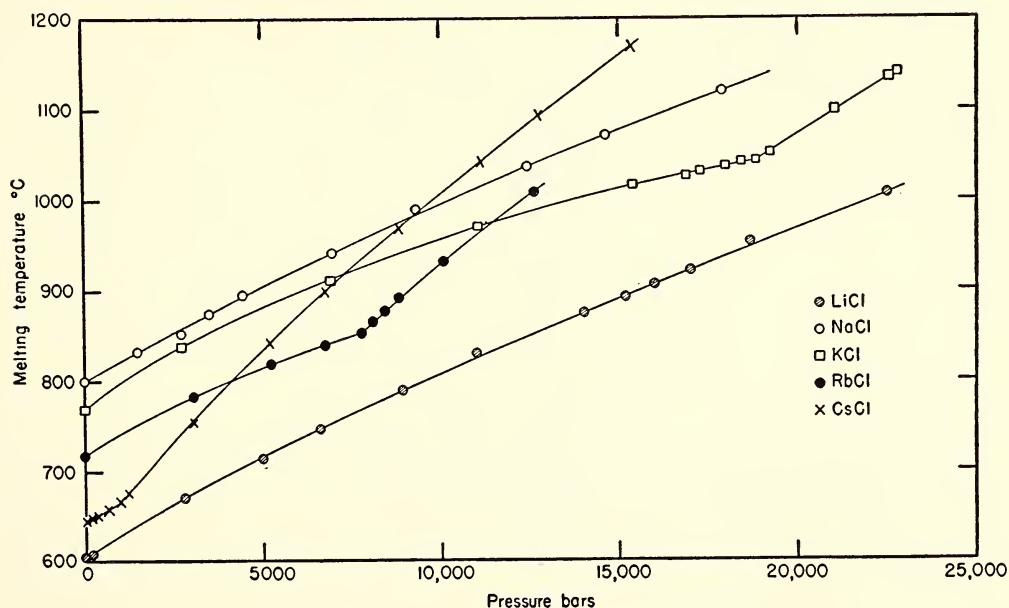


Fig. 4. Melting curves of the alkali chlorides.

chemical data. For these reasons, the initial experimental work has been confined to the alkali halides.

The melting curves of five alkali chlorides and four sodium halides were followed to a pressure of nearly 25,000 bars. The measurements were made in the high-pressure apparatus developed by Francis Birch at Harvard University. Sealed capsules containing the charges were packed around a thermocouple inside the pressure vessel, and melting and freezing were detected by arrests in heating and cooling curves.

Preliminary results for the eight salts are

shown in figures 4 and 5. The triple point of KCl may be particularly useful in the calibration of other types of high-pressure equipment at high temperatures.

The observed slopes of the melting curves at low pressure agree with the values calculated from Clapeyron's equation only for NaF. For the other salts the measured slope is always less than that calculated from thermochemical data; in some instances the discrepancy is as large as 50 per cent.

The cause of this discrepancy has not yet been determined, and the low-pressure ends of the melting curves are being re-

examined in a different apparatus. Results obtained with it to date are in good agreement with those found earlier, and indicate that the observations are reproducible and free of instrumental bias.

Other possible causes of the discrepancy are systematic errors in the thermochemical data arising from premelting phenomena,

and contamination of the salts used in the present work. The main contaminant is likely to be water, which is notoriously difficult to remove from alkali halides. Experiments are being continued with material which has been carefully dewatered, and they should aid in clarifying the situation.

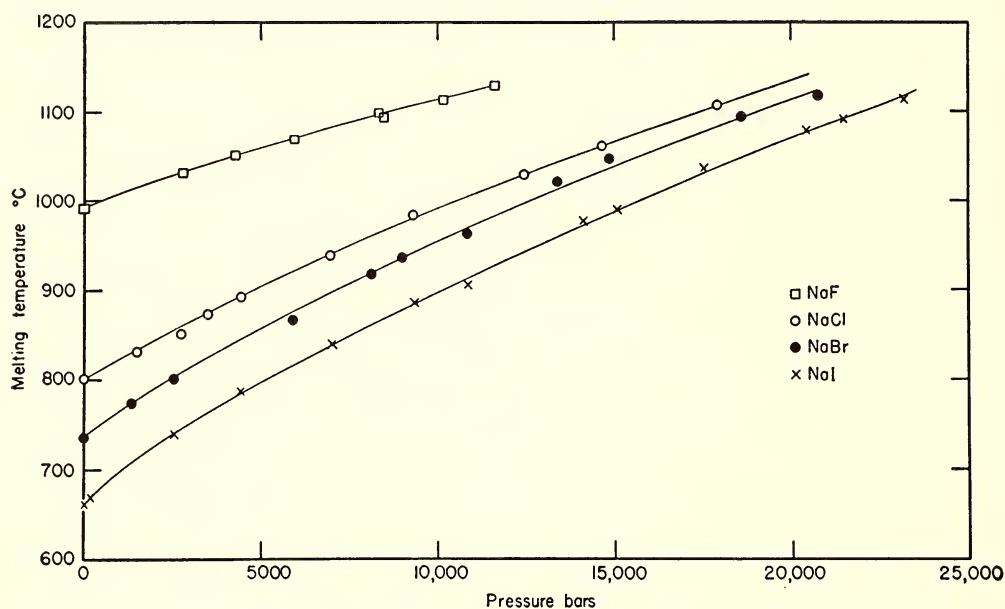


Fig. 5. Melting curves of the sodium halides.

THE AGE OF ROCKS AND MINERALS

(A cooperative program of the Geophysical Laboratory and the Department of Terrestrial Magnetism of the Carnegie Institution of Washington)

G. L. Davis, G. R. Tilton, L. T. Aldrich,¹ and G. W. Wetherill¹

Measurement of ages of coexisting minerals in a series of metamorphic rocks has revealed new opportunities for the application of dating methods. This was discovered first for zircon and biotite from the Baltimore gneiss, part of the basement complex of the Appalachian Piedmont. The zircon is 1100 million years old, on the basis of nearly concordant uranium-lead ages, and the biotite age is established as about 300 million years by rubidium-

strontium and potassium-argon methods. It appears that the gneiss crystallized 1100 million years ago, and that the biotite age is related to local metamorphism some 800 million years later. It has been established that rocks with ages of 1000 to 1150 million years, previously known in the southern Ontario and the Adirondack Mountains, are found in the Appalachian Mountain belt from the Catskills in New York to Shenandoah National Park, Virginia, increasing considerably the geographic extent of this group of rocks.

¹ Department of Terrestrial Magnetism.

Appalachian Ages

The Appalachian orogenic belt, which extends along the Atlantic coast of the United States and southeastern Canada, experienced several periods of deformation between 250 and 400 million years ago, during Paleozoic time. The process may be visualized as follows. Great thicknesses of sediments were deposited on a basement of gneisses and granites; both sediments and basement sank into the crust, where they were subjected to the effects of elevated temperature and pressure. In this environment some of the crystalline rocks and sediments became altered to metamorphic rocks. The metamorphism was accompanied by the formation or injection of granitic rocks. Later uplift and erosion exposed the resulting assemblage, including gneiss, schist, marble, quartzite, and granite. Such a process might be expected to produce rather complicated age patterns for some of the rocks. Several questions may be asked about this over-all process: (1) How old are the crystalline rocks of the basement complex? (2) What was the effect of Paleozoic metamorphism on the age of minerals in these rocks? (3) What was the sequence of the metamorphic events from one part of the belt to another? Did these events take place simultaneously along the whole belt?

The Appalachian orogenic zone is almost ideally suited for studies of this type. It is old enough so that erosion has produced good exposures of rocks of widely different ages. It is also old enough for daughter products to have accumulated from the various radioactive parents in sufficient quantity to allow accurate age determination. On the other hand, it is young enough to enable small time intervals of the order of 10 million years to be distinguishable by careful analytical work. In old Precambrian minerals differences of this order would be lost in analytical errors. Finally, many parts of the Appalachians are well mapped, and some stratigraphic control is available from fossil evidence.

The Appalachian age data are given in table 1. The first three entries—the Canada Hill gneiss, Storm King granite, and Mary's Rock gneiss—represent rocks having biotite and zircon with ages about 1000 million years. An age of 1000 to 1150 million years deduced from the zircon probably dates the time of crystallization of these rocks best. This value is based primarily on the $U^{235}\text{-Pb}^{207}$ and $Pb^{207}\text{-Pb}^{206}$ ages, which should be the more reliable ones. It is apparent that metamorphic events which took place 300 to 400 million years ago had little effect on the age record given by zircon and biotite. (The pattern of ages observed in these zircons could not be produced by removing lead from a much older zircon, for example, with an age of 1800 million years, in Paleozoic time since the two U-Pb ages would then be discordant.) Rocks with ages of 1000 to 1150 million years have long been known in the Canadian Shield in southern Ontario, and a zircon of this age has been found in the Adirondack Mountains. It now appears that rocks of this age extend along the Appalachian Mountain belt at least as far south as Shenandoah National Park. Long and Kulp, of the Lamont Geological Observatory, have measured Rb-Sr and K-A ages of about 900 million years on biotite near Hampton, Tennessee. This may be taken as a good indication that rocks of this age group are found even farther to the south.

In other parts of the belt the mica ages, and some zircon ages as well, were affected to a greater extent. Examples of these constitute the remainder of table 1. The results from the Baltimore gneiss are particularly important. Wasserburg, Petti-john, and Lipson have recently determined the K-A ages on micas from a number of metamorphic rocks, including the Baltimore gneiss and pegmatites in the Baltimore area. The values they observed were in the range from 300 to 350 million years, and established the time of most recent metamorphism of the rocks at about 330 ± 20 million years. Our values for Rb-Sr

and K-A ages for biotite from the Baltimore gneiss at Baltimore (table 1) are in this same range. Zircon from the Baltimore gneiss, on the other hand, gives nearly concordant and much older values, which are in the range observed at Bear

years ago; or the rock existed as an unconsolidated sediment with detrital zircon until 300 million years ago, then crystallized in such a manner as not to alter the zircon ages appreciably. Because the zircon and microcline ages agree quite well with

TABLE 1. Appalachian Ages

Location	Mineral	Age, million years					
		$\frac{U^{238}}{Pb^{206}}$	$\frac{U^{235}}{Pb^{207}}$	$\frac{Pb^{207}}{Pb^{206}}$	$\frac{Th^{232}}{Pb^{208}}$	$\frac{Rb^{87}}{Sr^{87}}$	$\frac{K^{40}}{A^{40}}$
Bear Mountain, N. Y.							
Storm King granite	Zircon	960	990	1060	850		
	Biotite					940	850
Canada Hill gneiss	Zircon	1140	1150	1170	1030		
	Biotite					900	780
Shenandoah National Park, Va.							
Gneiss, Mary's Rock Tunnel	Zircon	1070	1100	1150	1110		
	Biotite					890	800
Baltimore, Md.							
Baltimore gneiss							
Phoenix dome	Zircon	960	1020	1120	1100		
	Biotite					310	388
	Microcline					1200 \pm 200	
Towson dome	Zircon	1040	1070	1120	940		
	Biotite					305	338
	Microcline						308
Woodstock dome	Biotite					310	
Philadelphia, Pa.							
Baltimore gneiss	Zircon	1010	1050	1120	950		
	Biotite					390	550
Hibernia, N. J.							
Dark gneiss	Biotite					920	790
Light gneiss	Biotite					840	630
Spruce Pine, N. C.							
Cranberry gneiss	Zircon	1080	1140	1270	950		
	Biotite					350	322
Washington, D. C.							
Kensington gneiss, Sample A	Zircon	370	395	550		
	Biotite					305	380
Kensington gneiss, Sample B	Zircon	400	420	510	350		
	Biotite						350

Mountain and Mary's Rock. Microcline from the Baltimore gneiss at the Phoenix dome gives a Rb-Sr age that is in agreement with the zircon age; but it appears to have lost all its argon during Paleozoic metamorphism. Two interpretations may be given for this array of mineral ages—the rock crystallized 1100 million years ago and biotite was recrystallized 300 million

those found for zircon and biotite at Bear Mountain and Mary's Rock, and examination of thin sections indicates that a detrital origin for the microcline in the Baltimore gneiss is improbable, it is believed that the gneiss belongs to the 1000- to 1150-million-year group, and that it was sufficiently metamorphosed 300 to 350 million years ago to cause the microcline to lose

argon and the biotite to lose both strontium and argon.

The Rb-Sr age of 390 million years for the biotite in the Baltimore gneiss at Philadelphia may indicate an older biotite which did not lose quite all its strontium during metamorphism; this is so far the only mica age for this area, however. The higher K-A age is suggestive. Last year's report called attention to similar discordances in the ages of micas from certain metamorphic rocks from the Sudbury district, Ontario.² Thus, K-A ages higher than Rb-Sr ages appear to be characteristic of micas in some metamorphic rocks. The K-A age is somewhat higher than the Rb-Sr age for biotite in the Baltimore gneiss at Towson dome, possibly owing to the same effect and indicating that the Rb-Sr age is a better measure of the time of most recent metamorphism.

The Mary's Rock-Baltimore gneiss comparison is in agreement with field observation that the intensity of metamorphic processes varies across the orogenic belt, the rocks to the west being less affected than those of the Piedmont province to the east.

At Hibernia, New Jersey, only mica determinations have been completed. The Rb-Sr age of the biotite from the dark gneiss is similar to mica ages found at Bear Mountain and Mary's Rock. The ages for the mica from the light gneiss are lower and would indicate a higher degree of metamorphism. Both micas probably belong to the 1000- to 1150-million-year group, but the light gneiss was more highly metamorphosed 300 to 350 million years ago.

Results for the Cranberry gneiss resemble those from the Baltimore gneiss except that the zircon ages are discordant. The $\text{Pb}^{207}\text{-Pb}^{206}$ age of 1270 million years may indicate the presence of rocks older than the 1000- to 1150-million-year group

found farther north, and may be related to the occurrence of rocks with ages of 1300 to 1450 million years farther to the west (see fig. 6). The Rb-Sr age of 370 million years for the biotite agrees with ages established for both uraninite and muscovite from the Chestnut Flat pegmatite near Spruce Pine and appears to date a period of metamorphism.

Discordant ages are found in zircon from the Kensington gneiss, which indicate that the mineral was affected by the metamorphism that occurred 300 to 350 million years ago. The age data are consistent with the hypothesis that the zircon is 1100 million years old but lost about 90 per cent of its lead 300 million years ago.

Other Ages

Zircon from a gneiss at Koli, Finland, was also studied. The gneiss forms part of the basement complex in the Karelidic orogenic belt, occupying a position analogous to that of the Baltimore gneiss in the Appalachian belt. The zircon was supplied by Kouvo, who has measured the age of biotite from several gneisses in the area and found Rb-Sr and K-A ages of 1800 million years. The results are given in table 2. Unfortunately, the ages are discordant. The $\text{Pb}^{207}\text{-Pb}^{206}$ age suggests that the zircon is 2600 to 2700 million years old. Possibly the gneiss is 2600 to 2700 million years old and was metamorphosed 1800 million years ago. This may thus be another example of the effect observed for the Baltimore gneiss.

Although most of the central United States is covered with sediments, so that the crystalline basement rocks can be obtained only as drill cores, exposures of crystalline rocks do occur in the Arbuckle Mountains in southern Oklahoma and in the St. Francis Mountains and the Decaturville uplift in southern Missouri. Rubidium-strontium ages of about 1400 million years have been measured in these areas, and are given in table 2. Many micas with similar ages have been found

² This observation has been confirmed independently by measurements made at the Massachusetts Institute of Technology.

in the western United States (see fig. 6). The Wichita Mountains, 100 miles to the west of the Arbuckles, have a decidedly

sota, and Kenora, Ontario. When these results are combined with previous ones obtained here and in other laboratories—

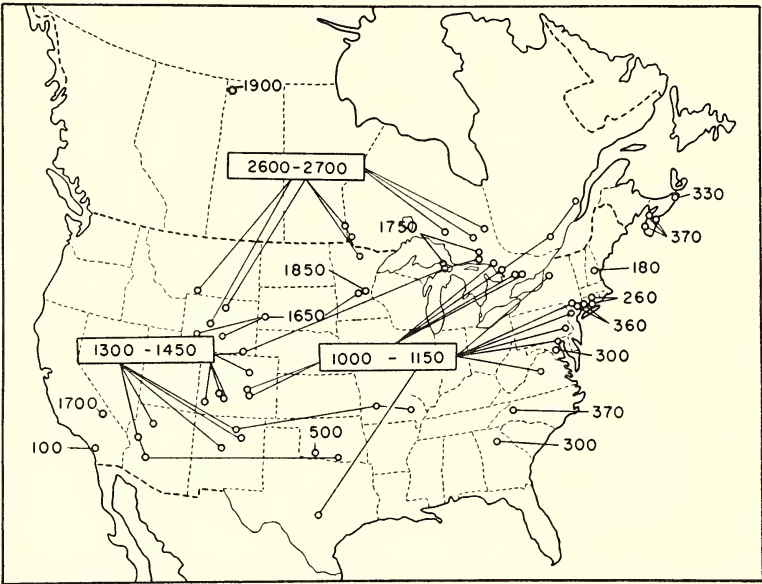


Fig. 6. Reliably dated localities in North America.

TABLE 2. Miscellaneous Ages

Location	Mineral	Age, million years					
		U ²³⁸	U ²³⁵	Pb ²⁰⁷	Th ²³²	Rb ⁸⁷	K ⁴⁰
		Pb ²⁰⁶	Pb ²⁰⁷	Pb ²⁰⁶	Pb ²⁰⁸	Sr ⁸⁷	Ar ⁴⁰
Koli, Finland (55 km north of Joensuu), gneiss	Zircon	1890	2270	2650	1790		
Troy, Okla. Ten Acre granite	Biotite					1360	
Decaturville, Mo. Pegmatite	Muscovite					1450	
Fredericktown, Mo. Einstein Mine	Muscovite					1450	1405
International Falls, Minn. Biotite knot in Vermilion granite	Biotite					2610	2630
Kenora, Ont. Granite	Biotite					2550	

younger age of 500 to 550 million years, as shown in last year's report.

Two new occurrences of micas with ages of around 2600 million years have been found at International Falls, Minne-

particularly those by P. W. Gast and L. E. Long, of the Lamont Geological Observatory, in the Bighorn Basin of Wyoming and Montana—a belt of rocks with an age of 2600 to 2700 million years, reaching from

central Ontario to western Wyoming and Montana, appears to have been defined. The locations of these rocks are given in figure 6. Ages of 2500 to 2700 million years have been determined by the K-A method at the University of Minnesota for mica in rocks from northern Minnesota and in the Minnesota River valley in the southern part of the state. Although Rb-Sr ages have not been completed, the argon work undoubtedly establishes the presence of rocks with ages of 2600 to 2700 million years in Minnesota.

North American Ages

Figure 6 shows the locations in North America where reliable mineral ages have been obtained. These values are based on samples for which at least two isotopic ages are in agreement, for example, the two U-Pb ages of a zircon or uraninite or the Rb-Sr and K-A ages of a mica. The only exceptions are the Arbuckle Mountains, Missouri, and Kenora samples, for which only Rb-Sr measurements have been completed. There are many other localities for which single Rb-Sr or K-A ages or discordant lead ages are available, but they have been omitted. The results shown include the work of several other laboratories.

Figure 6 shows rocks with ages of 2600 to 2700 million years extending probably as a belt from central Ontario to western Wyoming. To the south and southeast the rocks appear younger. It is now apparent

that rocks with ages of 1000 to 1150 million years are common in the Appalachian Mountains, uplifted during the Paleozoic era; and rocks with ages of 2600 to 2700, 1300 to 1450, and 1000 to 1150 million years are not rare in the region of the Laramide orogenic belt where deformation took place 60 million years ago.

It is apparent from figure 6 that most of the localities that have been measured lie south of the 2600- to 2700-million-year belt. Information north of the belt and around the ends is needed. When ages have been accumulated for the whole of the continent it may be possible to make a critical evaluation of its mode of formation. The question is whether the continent has grown from an old "nucleus" by addition of materials from depth during successive orogenies or whether it has always had considerable area.

Acknowledgments

C. A. Hopson and A. C. Waters (Johns Hopkins University) have provided valuable assistance in the collection of specimens of the Baltimore gneiss and other local rocks. Thanks are also due to W. E. Ham (Oklahoma Geological Survey) and C. A. Merritt (University of Oklahoma) for guidance in the collection of samples in the Wichita and Arbuckle Mountains in Oklahoma. W. C. Hayes (Missouri Geological Survey) and Clayton Johnson (University of Missouri) gave similar aid in the collection of samples from Missouri.

MINERAL ASSEMBLAGES IN THE GREEN RIVER FORMATION

H. P. Eugster and C. Milton³

Since the fundamental analysis of marine salt deposits by van't Hoff (1905, 1906), the mineral assemblages of such deposits have often been considered the best examples to demonstrate the principles governing the coexistence of minerals. The sequences of minerals obtained on evap-

oration from sea water and upon subsequent metamorphism as well as the associated reaction temperatures have become known through the efforts of a number of investigators. Least known today are some of the saline beds deposited from fresh waters, particularly those poor in chlorine.

The Green River formation of Wyoming, Utah, and Colorado contains such

³ U. S. Geological Survey, publication approved by Director.

saline beds, consisting chiefly of sodium carbonates, as well as a number of other unusual minerals. The beds containing saline minerals are intercalated between calcareous (dolomite and calcite) oil shale and detrital (often tuffaceous) beds and must have precipitated from vast lakes with extensive fluctuation in the water levels. The compositions of the lake waters were somewhat unusual, since chlorine, potassium, and sulfate ions played a very minor role.

The coexistence of certain minerals in the three major basins (Green River in Wyoming, Uinta in Utah, and Piceance Creek in Colorado) gives valuable insight into the general conditions of evaporation and precipitation as well as into local differences. Minerals considered are nahcolite (NaHCO_3), trona ($\text{NaHCO}_3 \cdot \text{Na}_2\text{CO}_3 \cdot 2\text{H}_2\text{O}$), shortite ($\text{Na}_2\text{CO}_3 \cdot 2\text{CaCO}_3$), eitelite ($\text{Na}_2\text{CO}_3 \cdot \text{MgCO}_3$), calcite (CaCO_3), dolomite ($\text{CaCO}_3 \cdot \text{MgCO}_3$), northupite ($\text{Na}_2\text{CO}_3 \cdot \text{MgCO}_3 \cdot \text{NaCl}$), analcite ($\text{NaAlSi}_2\text{O}_6 \cdot \text{H}_2\text{O}$), searlesite ($\text{NaBSi}_2\text{O}_6 \cdot \text{H}_2\text{O}$), reedmergnerite (NaBSi_3O_8), riebeckite ($\text{Na}_2(\text{Mg,Fe})_3\text{Fe}_2\text{Si}_8\text{O}_{22}(\text{OH})_2$), acmite ($\text{NaFeSi}_2\text{O}_6$), sepiolite ($\text{Mg}_2\text{Si}_3\text{O}_6(\text{OH})_4$), and loughlinite ($(\text{Na}_2,\text{Mg})\text{Si}_3\text{O}_6(\text{OH})_4$).

The spatial distribution of trona and nahcolite is most revealing. Trona occurs in Wyoming only and in a single bed 10 feet thick and covering several hundreds of square miles. Nahcolite is characteristic of the saline beds in Utah and Colorado and forms concretions and pockets inches to feet in diameter in a dolomitic matrix. The absence of nahcolite in Wyoming and of trona in Colorado and Utah is indicative of local differences between the evaporating basins.

Figure 7 shows a P_{CO_2} - T diagram for the system NaHCO_3 - Na_2CO_3 - H_2O at 1 atm total pressure, recalculated from data determined by Freeth (1923). The positions of the phase boundaries are not accurately known, since the calculations involve dissociation constants and activity

coefficients in very concentrated solutions. Determination of the phase boundaries by direct experiments is under way. Nahcolite occupies the area of high CO_2 content throughout, whereas soda (= natrite, $\text{Na}_2\text{CO}_3 \cdot 10\text{H}_2\text{O}$) is more characteristic of low temperatures. Trona is not stable below 19.7°C , the temperature of the isobaric invariant point nahcolite + soda +

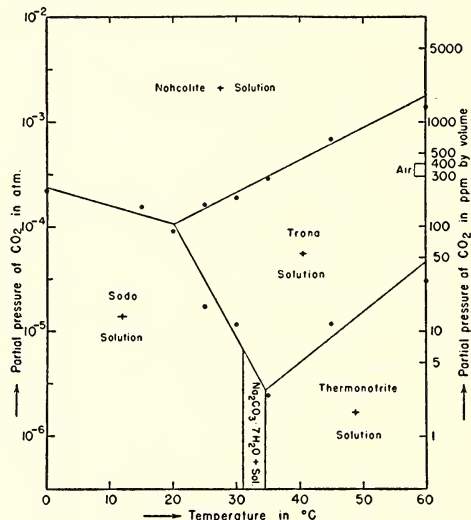


Fig. 7. P_{CO_2} - T diagram of the system NaHCO_3 - Na_2CO_3 - H_2O . The CO_2 content of present-day air has been indicated on the right. Locations of phase boundaries were calculated from the data of Freeth (1923).

trona + solution. Thermonatrite will form only above 34°C and at low CO_2 pressures. The soda-trona boundary is very steep; hence the occurrence of soda rather than trona is primarily a function of temperature. In some lakes trona precipitates during the warm seasons and soda during the colder ones.

For the saline beds of the Green River formation the location of the trona-nahcolite boundary is critical. As a point of reference the CO_2 content of present-day average air (300 to 400 ppm) has been indicated on figure 7. A solution of sodium carbonates equilibrated with such air will precipitate on evaporation both nahcolite and trona at a temperature of about 36°C .

The occurrence of trona in Wyoming rather than nahcolite must be due to higher temperatures or lower CO_2 pressures, or both, than those characteristic of the Utah and Colorado basins.

The concentrations of the brines necessary to precipitate sodium carbonates give

of trona alone requires even greater concentrations. From a comparison of figure 8 with figure 7 it becomes clear that the highest concentrations of Na^+ are required to saturate solutions with the lowest CO_2 pressures. At very high CO_2 pressures, that is from solutions virtually devoid of

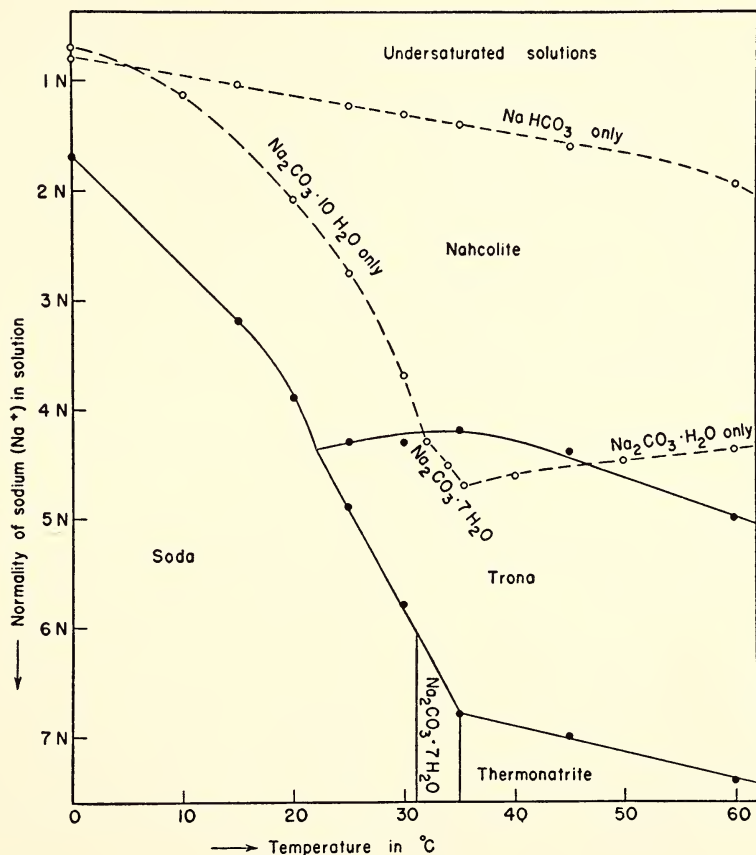


Fig. 8. Concentration of sodium ion in solution as a function of temperature. Solid lines are for the system $\text{NaHCO}_3\text{--Na}_2\text{CO}_3\text{--H}_2\text{O}$ as determined by Freeth (1923). Broken lines are for the systems $\text{NaHCO}_3\text{--H}_2\text{O}$ and $\text{Na}_2\text{CO}_3\text{--H}_2\text{O}$, respectively (*International Critical Tables*).

some further clues. Figure 8 shows the normality in sodium ions of saturated solutions as a function of temperature for the systems $\text{Na}_2\text{CO}_3\text{--H}_2\text{O}$, $\text{NaHCO}_3\text{--H}_2\text{O}$, and $\text{NaHCO}_3\text{--Na}_2\text{CO}_3\text{--H}_2\text{O}$. The solid field boundaries correspond to those of figure 7. Nahcolite and trona coprecipitate at normalities between 4 and 5, depending on the temperature, whereas the formation

$\text{CO}_3^{=}$ ions, nahcolite precipitates at very much lower concentrations of Na^+ (1 to 2 normal), as indicated by the curve labeled " NaHCO_3 only."

The brines from which trona precipitated in Wyoming must have been very concentrated, whereas evaporation in Utah *may* have been much less extensive. In Wyoming saturation occurred simultane-

ously over a very wide area, probably a shallow and warm pool, equilibrated with air. Average temperatures were higher than 35°C if the CO_2 content of Eocene air was similar to that of present-day air. These temperatures are reasonable since modern briny lakes have been found to be as hot as 56°C . In the Utah and Colorado basins the brines were probably covered with fresh water before saturation was reached. As a consequence of the increased depth of the lakes the temperature of the brines, which were preserved in the bottom layers, was lowered, while the CO_2 content increased owing to both greater pressure and slower exchange of biogenic CO_2 with the atmosphere. Calcium carbonate muds, which began to precipitate, isolated the brines into pockets, from which on further desiccation nahcolite had to precipitate. Hence the fundamental difference

between the Wyoming and the Utah and Colorado basins may have been simply one of depth.

The trona-nahcolite problem is only one of a great number of similar questions raised by the unusual mineral assemblages of the Green River formation. Little or no information is yet available about conditions under which such carbonates as shortite, eitelite, dawsonite, burbankite, pirssonite, gaylussite, bradleyite, and northupite and such silicates as analcite, searlesite, reedmergnerite, acmite, riebeckite, sepiolite, loughlinite, garrelsite, leucosphenite, elpidite, and labuntsovite can exist. They all must have formed at or near room temperature and probably under equilibrium conditions. The interpretation of their associations will eventually give us direct insight into the history of a group of ancient and very complex lakes.

GEOCHEMISTRY OF ARTIFICIAL ISOTOPES

W. F. Libby

The Beneficiation of Soils Contaminated with Strontium 90; Beneficial Effects of Potassium

There appears to be a possibility that part of the strontium in soils, like calcium, exists in forms unavailable to plants and thus to the biosphere. Evidence from the fallout data of the Sunshine Project disclosed disparities between total fallout as judged by actual pot collection of rain and the plant contents and soil analyses which could be due to some type of chemical aging or to development of chemical inaccessibility by the radiostrontium carried in the rain. Experiments by several investigators have shown that as much as 30 per cent of the radiostrontium in soils is not accessible to plants.

These results indicate the possibility of a beneficiation of heavily contaminated soils by the use of ordinary fertilizers in reasonable amounts. The consequences of reactor accidents or local fallout during wartime might thus be reduced consider-

ably. It seemed reasonable that the formation of certain insoluble inorganic compounds like strontium sulfate might produce such effects. Strontium sulfate occurs in some soils as the mineral celestite, and it might be expected to be sufficiently insoluble to accomplish at least a partial segregation of soluble strontium introduced into the soil. It is so insoluble (solubility product 7.6×10^{-7} at 25°C) that it seemed likely that in contrast to gypsum, $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$, with a solubility product of 2.4×10^{-5} at 25°C —which apparently can feed calcium into plants—strontium sulfate strontium might be truly unavailable to plant life. On the other hand, similar considerations on barium have been tested by Bradfield (1932), and Robinson, Whetstone, and Edgington (1950), and their results show that barium sulfate, which is even less soluble than strontium sulfate, can be utilized by plants in certain soils. Since, however, the possibility seemed to exist that the addition of sulfate to con-

taminated soils might be helpful, an investigation was undertaken. Because certain earlier work had indicated that potassium might have a considerable beneficial effect on radiostrontium absorption, a search for a specific potassium effect was undertaken also. This note is a report of the experiments to test these two theories.

Soil from Washington, D. C. (garden at Geophysical Laboratory), was mixed, 2 parts to 1 of the commercial soil thinner Vermiculite and 1 part of horse manure fertilizer. The soil used to make the mixture had 32 milliequivalents of exchangeable calcium per 100 g. To about 2 pounds of this mixture was added in very dilute

Pot B was prepared in exactly the same way except that no sulfate was added. After the first crop, 265 mg of potassium nitrate was added to test the potassium effect. Pot C had no additions whatsoever except the tracer radiostrontium; it served as a control. Pot D was filled with the pure soil, unfertilized and untreated with Vermiculite. To it was added radioactive strontium as the solid, insoluble strontium sulfate, 690 mg of the radioactive strontium sulfate being used to 726 g of soil, the two being intimately mixed before planting of the radish seeds.

The pots were planted with radish seeds and cultivated by setting in the ground

TABLE 3. Effect of Sulfate and Potassium Treatment of Radiostrontium-Contaminated Soils on the Availability to Radish Crops

Pot	Conditions	Sr ⁹⁰ Content of Radish Ash Carbonates, arbitrary units †
Pot A (370 g mixture of soil and Vermiculite)	8.9 mg Sr/100 g as nitrate+9.5 mg K ₂ SO ₄ /100 g	0.63
Pot A (370 g mixture of soil and Vermiculite)	Above + 22 mg Sr/100 g as nitrate + 50 mg K ₂ SO ₄ /100 g	0.64, 0.58
Pot B (356 g mixture)	9 mg Sr/100 g as nitrate	0.81
Pot B (356 g mixture)	Above + 72 mg KNO ₃ /100 g	0.62, 0.63
Pot C (380 g mixture)	No additions, except tracer Sr *	1.00, 1.01, 0.98
Pot D (726 g soil only)	43 mg Sr */100 g as Sr *SO ₄	0.90

† Each entry is one crop.

aqueous solution approximately 10 microcuries of strontium 90. Four earthen pots were used for trial with radish seeds for test of the efficacy of the purposeful addition of SO₄⁼ and K⁺ in the reduction of plant pick-up of the radiostrontium.

Pot A containing 370 g of the contaminated soil mixture was prepared as follows: Within a few minutes after the addition of the radiostrontium to the soil, 32 mg of ordinary nonradioactive strontium was added in dilute aqueous solution as nitrate. The soil was stirred and made into a thick mud by further addition of water. After about 15 minutes, 35 mg of K₂SO₄ was added in dilute aqueous solution and stirred. After one crop, another 81 mg of strontium as nitrate and 200 mg of K₂SO₄ were added in the same manner.

outside in the open during the summer or by exposing to a bank of fluorescent lights indoors in the winter. At maturity the plants were ashed (after careful washing), the ash was dissolved in dilute hydrochloric acid, and sodium carbonate solution was used to precipitate the insoluble hydroxides and carbonates, which were measured for strontium 90 content. The results are presented in table 3.

The results indicate clearly that the addition of sulfate is not very effective as a means of reducing radiostrontium pickup by crops grown on contaminated soils. Although additional soluble strontium does seem to have some effect, the principal one was caused by potassium, for which, at as low a level as about 60 pounds per 2 million pounds of soil (or about 30 pounds

per acre for normal 2-inch depth of penetration of water-soluble fallout), something like a 40 per cent reduction of radiostrontium uptake was observed.

Although these experiments show that radish plants on certain kinds of soil certainly can utilize the strontium in strontium sulfate, and that the formation of radiostrontium sulfate does not necessarily reduce the uptake of radiostrontium, the positive effect of potassium is established. It is possible that additional fertilizers or amendments may have a more marked effect than either of the two investigated in this work.

The partial retention of radiostrontium in soils may involve effects other than those tested here. Certainly, as strontium remains in the soil it is very likely eventually to be incorporated into large crystals, where it will become physically unavailable to the plants. And so the possibility of chemical aging, taking place slowly over several years, exists. It does not seem likely, however, that this process will take place on a large enough scale to return heavily contaminated soil to a useful condition, and further work needs to be done on methods of quick beneficiation.

Rain Fallout

During the past year, samples of rain have been collected regularly to determine the radioactive fallout content. A wash-tub (2.5 square feet area) is placed out in the open; the water is bottled and brought into the laboratory for processing. Larger samples have also been collected from a roof. A rain gauge is used to determine the rainfall in each specific instance.

It has been well established that radioactive fallout is mainly carried down by rain and snow, the fraction deposited directly being minimal. Therefore, by sampling the rainfall and snowfall, it is possible to determine the radioactive fallout in a given area. The purpose of the work in the Laboratory has been to settle a particular point about the nature of the atmospheric storage mechanism.

In the model proposed by the author, the fallout material introduced into the stratosphere is immediately mixed horizontally to a uniform concentration and has a residence time of about 10 years there; that is, about 10 per cent of the material resident in the stratosphere precipitates annually. The point of interest is whether this simple theory can actually explain the amount of radioactive fallout observed at a given locality and, more important, its composition in terms of the short-lived isotopes; in other words, can it explain the age of the fallout, the length of time that it has been airborne before being reprecipitated.

According to this simple model, stratospheric material is airborne for about 10 years and tropospheric material for only 1 month on the average. Therefore, if during periods of heavy precipitation heavy fallouts were observed to contain relatively little of the short-lived isotopes, it would show that the stratospheric material was coming down and at a variable rate which could not be maintained throughout the whole year. Some meteorologists, particularly Lester Machta of the U. S. Weather Bureau, have stated that meteorological considerations of stratospheric wind patterns have led them to the conclusion that heavier fallout should be observed in the general latitude of 40° to 50° N. and similarly in the southern hemisphere at certain seasons of the year. Therefore, they predict that the heavier fallout observed in the spring and summer of each year in our latitudes is mainly of stratospheric origin. The author's model predicts that the increased fallout would be of tropospheric origin, and of younger age than debris of stratospheric origin. The question of which model is correct is an important one, since it obviously affects the amounts of fallout to be expected in the populous latitudes of the northern hemisphere.

The procedure for settling the point is straightforward. Among the fission products is barium 140, which has a half-life of 12.8 days and is therefore appropriate for

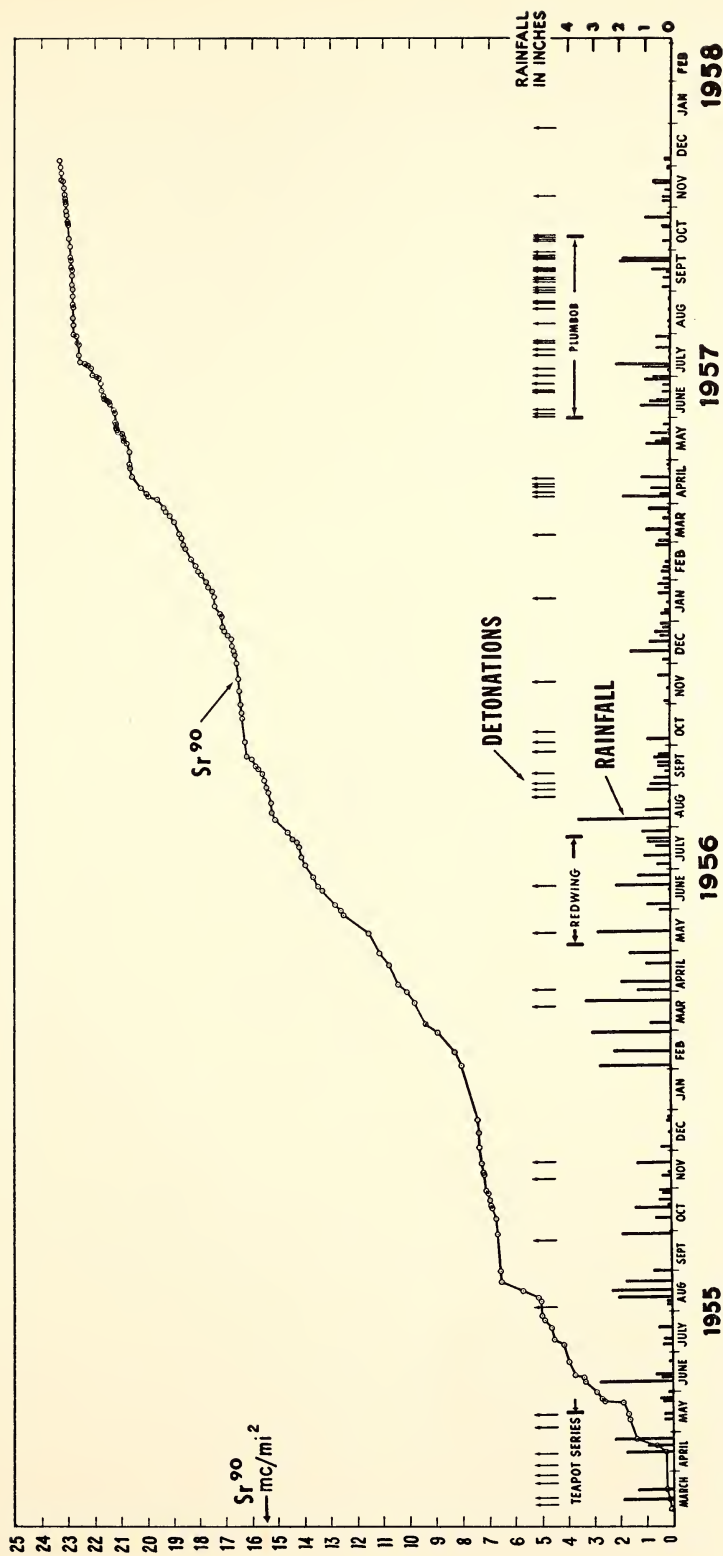


Fig. 9. Pittsburgh Sr^{90} fallout, 1955-1958; galvanized-tub collections.

distinguishing between fallout ages of 1 month and about 1 to 2 years. The radiochemical procedure for barium 140 is similar to that for strontium 90, and both are more sensitive and reliable than that for

10 gives the data obtained so far in the analysis of the Geophysical Laboratory samples in Washington, D. C. They are incomplete, but they do show an interesting point: that during the months of

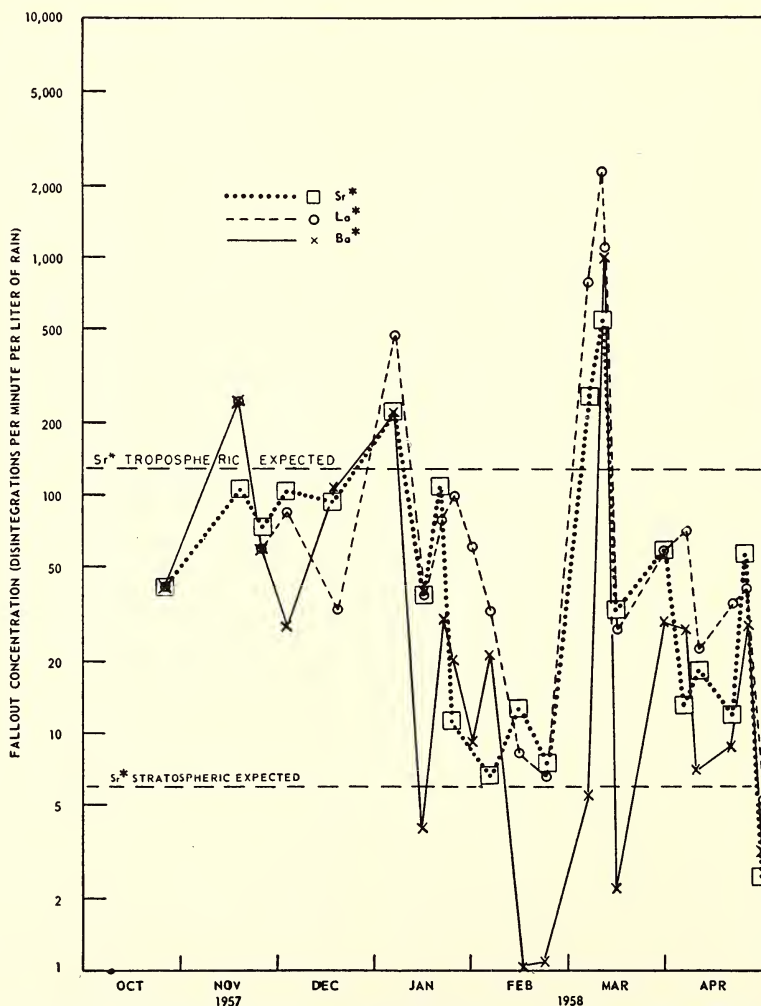


Fig. 10. Rain fallout in Washington, D. C. Ba*, La*, and Sr* separated chemically and measured 2 weeks after collection.

the 51-day strontium 89 isotope, which is particularly susceptible to errors and radioactive impurities. Figure 9 shows the type of variation of strontium 90 fallout observed at Pittsburgh. Presumably, the Washington, D. C., samples, when fully analyzed, will give a similar curve. Figure

February, March, and April 1958 relatively little young fallout came to Washington. Therefore, these samples are particularly important for determining whether the rise that has normally been observed in the spring in the past years will occur this year.

Tritium Hydrology

Investigations already made have indicated the usefulness of tritium in the study of such problems as the circulation of ground water, the circulation of water masses in the ocean, and atmospheric circulation, as well as in cosmic-ray research. Another application of tritium to hydrology and geophysics is the use of synthetic tritium water to label rather extensive areas for hydrological and geophysical purposes. Radioactive water could be distributed either by low-flying airplanes, by drilling and injection, or by sprinkler systems over a given area that is being investigated hydrologically or geophysically. This type of experiment is planned in collaboration with Luna B. Leopold, Chief Hydraulic Engineer, U. S. Geological Survey. Mr. Lee Thatcher, of the Survey, is making hydrologic studies with cosmic-ray and bomb tritium.

The counter and apparatus for measuring tritium in electrolytically enriched water samples have been installed and are

functioning well; they are part of the apparatus used at the University of Chicago and brought to the Laboratory some time ago.

The principal problem being attacked at the present with the equipment is the measurement of stratospheric water for tritium content. Stratospheric water samples, collected in connection with a balloon sampling program for fallout materials, are being measured for tritium to observe whether the bomb tests have appreciably contaminated the stratosphere with tritium. It is expected that they will not show very serious contamination, because the bomb tritium is immediately burned to water and is diluted with enormous masses of ordinary tropospheric moisture, which, when the bomb cloud reaches the stratosphere, condenses into ice crystals in the cool upper air and falls back into the troposphere in a matter of a few days. This is in sharp contrast to the fission products, which remain airborne for years when the cloud reaches into the stratosphere.

EXPERIMENTAL PETROLOGY

EFFECT OF WATER ON THE MELTING OF SILICATES

H. S. Yoder, Jr.

Most magmas contain some water, and it has been shown that water is an important factor in magmatic processes. In recent Year Books, for example, the large shift in the composition of the "eutectic" of simple rock-forming mineral systems under high water pressure was depicted (Yoder, Year Book 53, p. 107; Stewart, Year Book 56, p. 215). In addition, it was shown that water greatly expands the region in which crystals are in equilibrium with liquid in basalts (Yoder and Tilley, Year Book 55, p. 150). In other systems the effect of water on the liquid phase was elucidated under conditions where a gas phase is prohibited (Yoder, Stewart, and J. R. Smith, Year Book 55, p. 208). These and other effects are important in determining the course of events in a crystal-

lizing magma. Above all, however, the most significant role of water is the great lowering of the liquidus temperature of minerals and mineral systems. At H_2O pressures corresponding to depths of only 12 miles, the lowering of the liquidus may be 100°C ($\text{CaMgSi}_2\text{O}_6\text{-H}_2\text{O}$) or as great as 700°C ($\text{NaAlSi}_3\text{O}_8\text{-H}_2\text{O}$)! Data obtained to date by various workers on the melting behavior of common rock-forming silicates under high water pressure are summarized in figure 11.

In addition to the great lowering of melting temperatures by water, the effect of water on polymorphism and incongruent melting can be seen from the figure. In the $\text{SiO}_2\text{-H}_2\text{O}$ and $\text{NaAlSi}_3\text{O}_8\text{-H}_2\text{O}$ systems the high-temperature polymorphs are no longer stable at elevated water pressures. The fields of cristobalite and tridymite in the $\text{SiO}_2\text{-H}_2\text{O}$ system are successively suppressed with increasing water

pressure so that quartz melts directly to a liquid saturated with water. In a similar way the field of carnegieite in the $\text{NaAl-SiO}_4\text{-H}_2\text{O}$ system is suppressed so that a form of nepheline melts directly to a water-saturated liquid. In the $\text{KAlSi}_3\text{O}_8\text{-H}_2\text{O}$ system the incongruent melting behavior of high-sanidine to leucite and liquid is suppressed and high-sanidine melts directly to a water-saturated liquid. This

The lowering of the melting temperatures of these common rock-forming minerals with increasing water pressure also appears to support the idea advanced by Morey (1922) that very high pressures may develop during the cooling of a contained hydrous magma. If certain conditions are met, according to Morey, the hydrous magma on cooling will follow a univariant curve similar to those in figure 11. As

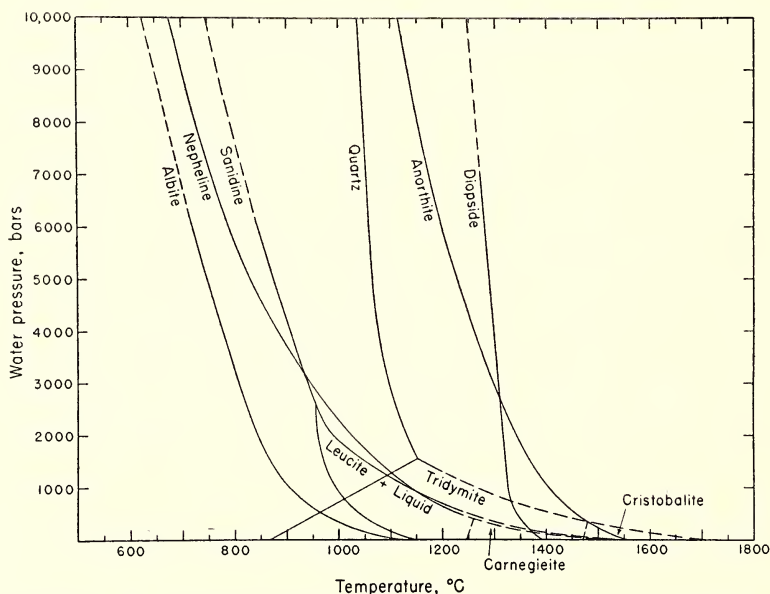


Fig. 11. Projection of univariant liquidus curves in portions of the following systems: (1) $\text{SiO}_2\text{-H}_2\text{O}$: Tuttle and England (1955); Yoder (unpublished, 1955); Stewart (unpublished, 1957). (2) $\text{CaAl}_2\text{Si}_2\text{O}_8\text{-H}_2\text{O}$: Yoder (unpublished, 1953); Stewart (unpublished, 1957). (3) $\text{NaAlSi}_3\text{O}_8\text{-H}_2\text{O}$: Yoder (unpublished, 1958). (4) $\text{KAlSi}_3\text{O}_8\text{-H}_2\text{O}$: Goranson (1938); Bowen and Tuttle (1950); Yoder, Stewart, and J. R. Smith (unpublished, 1957). (5) $\text{NaAlSi}_3\text{O}_8\text{-H}_2\text{O}$: Goranson (1938); Bowen and Tuttle (1950); Yoder (unpublished, 1953); Yoder, Stewart, and J. R. Smith (unpublished, 1957). (6) $\text{CaMgSi}_2\text{O}_6\text{-H}_2\text{O}$: Yoder (unpublished, 1953).

suggests that the effect of water on the melting temperature of leucite is more drastic than on that of sanidine.

One of the important discoveries in this study is that the melting temperatures of the rock-forming silicates in the presence of water are well within those believed to exist in the crust. The observations support the view that magmas exist in the crust, which on cooling would consist solely of highly refractory minerals such as anorthite, the principal constituent of anorthosites.

the temperature falls the pressure of gas in equilibrium with crystals and liquid rises sharply. Morey further predicted from his analysis of salt solutions and the system $\text{K}_2\text{SiO}_3\text{-SiO}_2\text{-H}_2\text{O}$ that the pressure would rise to a maximum and then decrease with further cooling. It appears from the data in the figure that this maximum pressure, if it obtains, exceeds that compensated by the weight and strength of the crustal rocks, approximately 10,000 bars at the base of the crust 35 kilometers in depth. If the melt curves continue to rise to high pres-

tures with cooling, it would be necessary to conclude that all contained hydrous magmas in the crust must eventually break the containing strata on cooling and intrude the country rock or extrude onto the surface of the earth. Geologic evidence indicates, however, that many magmas believed to have contained H_2O cool without greatly deforming their chamber or breaking out to the surface. Because of the discrepancy between conclusions drawn from laboratory and field observations, let us examine more closely the conditions required by Morey's theory.

It is assumed by Morey that the walls of the magma chamber are essentially rigid and impermeable. But rocks are known to vary in permeability, and some undergo deformation readily. It is possible, therefore, that the gas pressure may be relieved by diffusion of some of the gas through the walls of the chamber, by the formation of a hydrous phase, or by enlargement of the chamber itself. Perhaps the most important condition to be met for pressure generation is that the magma must reach a univariant condition in the early stages of cooling. That is, the magma can have only one degree of freedom as prescribed for the univariant curves of the simple binary systems shown in figure 11. If the magma is not univariant, the vapor pressure could be relieved by changing the relative proportion of crystals, liquid, and vapor. Recent experiments on basalts and granites suggest that the condition of univariancy is met early in the cooling of a relatively dry magma: the principal phases in both these major magma types appear together within a very narrow temperature interval when only several per cent of crystals are present. With increasing water content, however, the principal phases appear together within a much larger temperature interval, and the condition of univariancy is probably met only in the final stages of cooling. The restrictions of a rigid, impermeable magma chamber and the condition of univariancy do not appear to be easily met in a cooling complex magma.

Two other factors must be considered. It is possible that the melt curves are interrupted by critical phenomena or by phase changes. Critical phenomena have not been expected in magmas, according to Morey, because of the large number of components and the concentration of the more volatile components in the residual liquid. The less complex magmas, however, may exhibit critical phenomena in the light of the data on common rock-forming minerals. When the critical point is reached, the magma loses a phase and becomes divariant. The pressure may then be compensated by changes in the relative proportions of crystals and gas. Phase changes may be anticipated in magmas containing analcite, nepheline, and quartz, which are known to have high-pressure polymorphs. The formation of an additional phase will decrease the degrees of freedom. These factors will influence pressure generation.

The explosive volcanoes attest to the fact that the special conditions for pressure generation are met in magmas of a wide range of composition, yet it is not expected from the arguments stated above that the conditions will necessarily be met in the majority of hydrous magmas solely by cooling. External forces, causing a rise of the magma to a lower pressure region, for example, may be the principal agent attending the explosive release of water in magmas.

IRON-RICH CHLORITES

A. C. Turnock and H. P. Eugster

Of the group of layered silicates (micas, chlorites, clay minerals) the chlorites have received the least experimental attention. Yoder's work on clinocllore (1952) is the only quantitative information available on the stability of a chlorite. Yoder also was first to point out the polymorphic relations between the 7 Å phases (kaolinite types) and the 14 Å phases (true chlorites). Since most natural chlorites contain considerable amounts of iron, it was thought advisable to study the phase relations of

iron-rich chlorites as well. Daphnite, $5\text{FeO} \cdot \text{Al}_2\text{O}_3 \cdot 3\text{SiO}_2 \cdot 4\text{H}_2\text{O}$, the iron analog of clinocllore, was chosen as an example.

A 7 Å daphnite was synthesized from a variety of starting materials at temperatures as low as 400° C. Even at pressures as high as 5000 bars it has not been pos-

+quartz at intermediate P_{O_2} , and magnetite+mullite+quartz at high P_{O_2} . Gedrite was formed in several runs, but was shown to be a metastable product. Iron cordierite was also encountered between 600° and 650° C. Its position in the equilibrium diagram is not yet established.

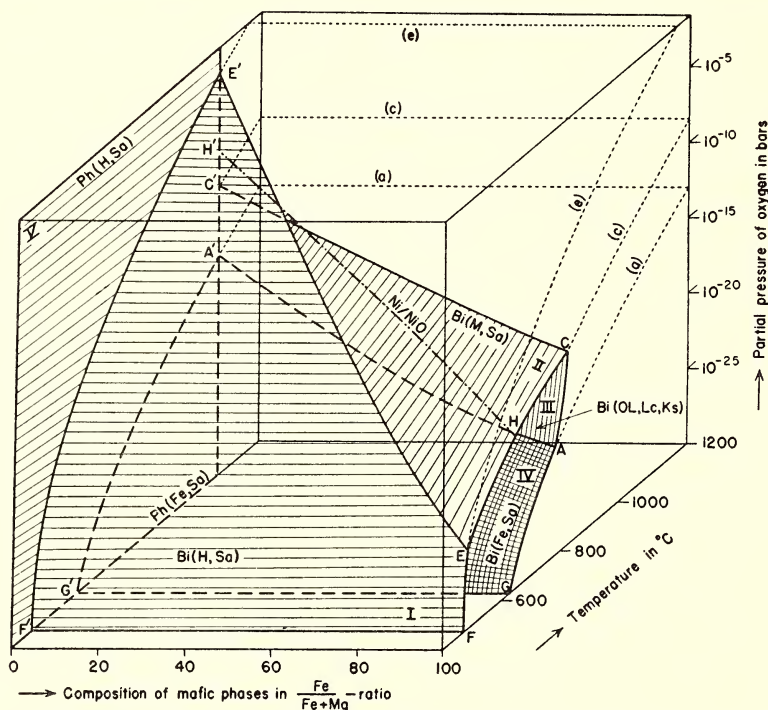


Fig. 12. Phase relations of biotites on the join phlogopite-annite at a constant pressure ($P_{\text{tot}} = P_{\text{H}_2\text{O}} + P_{\text{H}_2} = 2000$ bars). Ph, phlogopite; Bi, biotite; H, hematite; M, magnetite; Fe, iron; Sa, sanidine; Ol, olivine; Lc, leucite; Ks, kalsilite; Ph(H,Sa), locus of all phlogopites coexisting with H + Sa, and so on. Only surfaces representing micas coexisting with other phases have been drawn (I to V). The surfaces conjugate to surfaces I to V have been omitted.

sible to convert this form wholly to the 14 Å polymorph. P_{O_2} was controlled in all experiments employing the usual oxygen buffers. At 2000 bars water pressure the highest temperatures at which daphnite was synthesized for a series of oxygen pressures are as follows: 525° C (hematite-magnetite buffer), 600° C (quartz-magnetite-fayalite buffer), 650° C (magnetite-wüstite buffer). The following high-temperature assemblages were found on the daphnite composition: fayalite+hercynite+quartz at low P_{O_2} , magnetite+hercynite

BIOTITES

The mica biotite is one of the most common iron-containing minerals. The phase relations of two important end members of the biotite group have already been clarified: phlogopite, the magnesian mica (Yoder and Eugster, 1954); and annite, which contains ferrous iron (Eugster, Year Book 56). A third end member, a ferrous-ferric biotite, is now under investigation. Unquestionably the most important substitution in the biotite group is that of Fe^{+2} for Mg^{+2} . It is accompanied by major

changes in physical properties as well as stability.

Since annite is the best-known iron-rich end member of the biotites, it will be most fruitful to consider the phase relations on the join phlogopite-annite. Such an analysis, with appropriate modifica-

positional variations can be treated most successfully by plotting $\text{Fe}/(\text{Fe}+\text{Mg})$ ratios of the phases concerned.

Figure 12 shows a three-dimensional section with $P_{\text{H}_2\text{O}}$ fixed at 2000 bars ($P_{\text{tot}} = P_{\text{H}_2\text{O}} + P_{\text{H}_2} = 2000$ bars) for the join phlogopite (left)-annite (right). The curves (a),

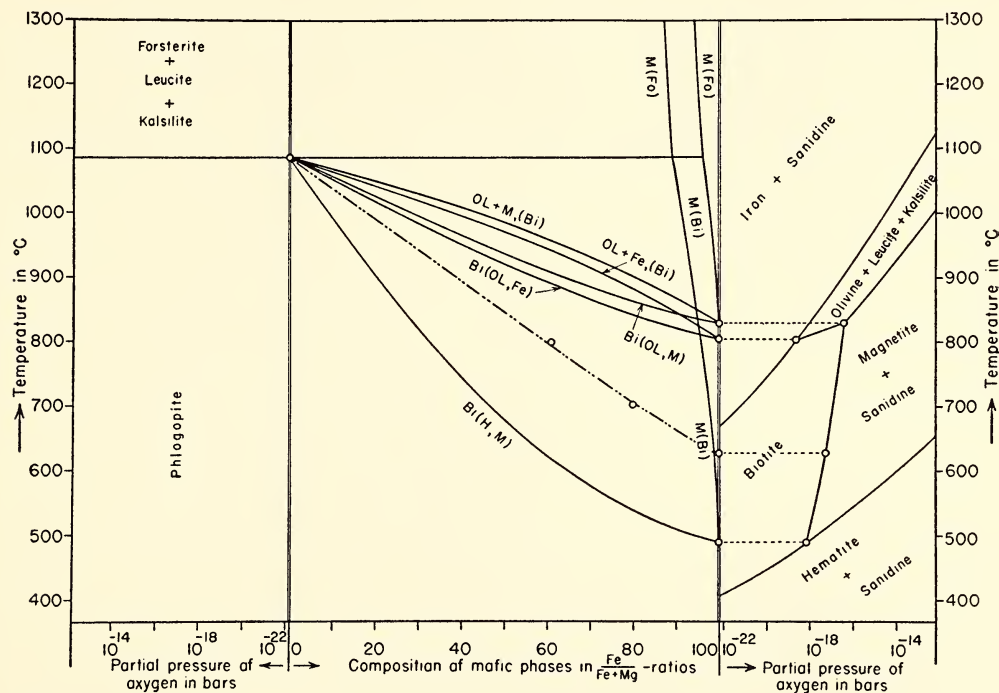


Fig. 13. T - X projection of the phase relations on the join phlogopite-annite at a total pressure of 2000 bars ($P_{\text{tot}} = P_{\text{H}_2\text{O}} + P_{\text{H}_2}$) projected along the P_{O_2} axis. P_{O_2} - T data for the end members phlogopite (left) and annite (right) have been rotated into the same plane. Projections of the intersections of conjugate surfaces onto the T - X plane have been labeled for Mg-Fe phases only, omitting feldspars and feldspathoids. Data for biotites at an oxygen pressure of a Ni + NiO buffer are dashed and labeled [Ni/NiO]. Abbreviations as in figure 12.

tions, is equally applicable to all Mg-Fe solid solutions and can be carried out using the data on the end members only.

Phase Relations of Hydrous Silicates with Intermediate Mg/Fe Ratios

H. P. Eugster and D. R. Wones

All iron-bearing members of Mg-Fe solid solutions are affected by changes in the partial pressure of oxygen. Consequently P_{O_2} , $P_{\text{H}_2\text{O}}$, P_{tot} , and T must be considered as independent variables. Com-

(c), and (e) on the right and left sides of the block diagram represent the reactions iron + oxygen \rightleftharpoons wüstite (a), wüstite + oxygen \rightleftharpoons magnetite (c), and magnetite + oxygen \rightleftharpoons hematite (e). Their position is essentially that given by Darken and Gurry (1945) with appropriate corrections for total pressure. The right side panel gives the data for annite (see Year Book 56, p. 162, fig. 11), whereas phlogopite on the left is stable up to a vertical line going through points A' , C' , and E' at 1085°C .

Between these panels a volume is represented within which biotites are the stable phases. This volume is bounded by the four curved surfaces I, II, III, and IV. I is defined by $FEE'F'$, II by $ECC'E'$, III by $CAA'C'$, and IV by $GAA'G'$.

Biotites lying on these surfaces can coexist with four different assemblages as follows: I, biotites coexisting with hematite + sanidine [Bi(H,Sa)]; II, biotites coexisting with magnetite + sanidine [Bi(M,Sa)]; III, biotites coexisting with olivine + leucite + kalsilite [Bi(OL,Lc,Ks)]; IV,

In order to construct figure 13 the curves EE' , CC' , and AA' of figure 12 have been projected onto the base. Simultaneously the left and right sides of the block diagram have been rotated into the same plane, giving the data for phlogopite in the left panel and those for annite in the right one. The $Fe/(Fe+Mg)$ ratios of the phases that coexist with biotites along the above curves have been added in figure 13. Data for the Ni/NiO buffered runs have also been plotted. Figure 13 is useful for presenting experimental results, but very

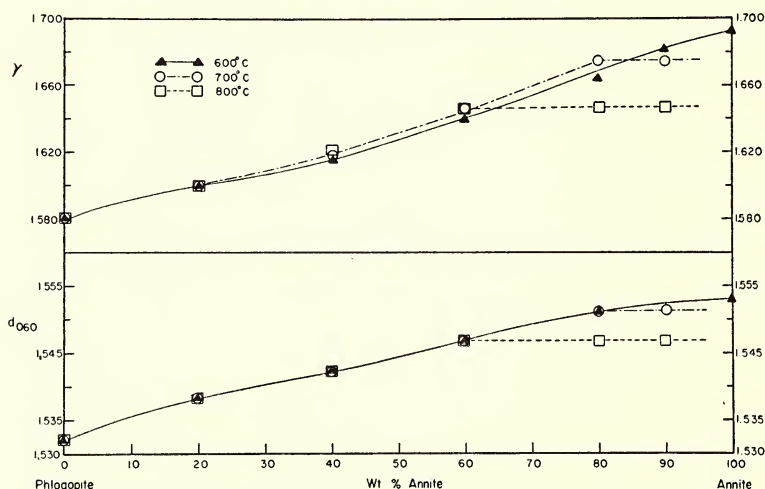


Fig. 14. Plot of index of refraction and $d(060)$ value versus composition for biotites on the join phlogopite-annite synthesized at several temperatures and oxygen pressures of a Ni-NiO buffer.

biotites coexisting with iron + sanidine [Bi(Fe,Sa)].

Figure 13 indicates that biotites can coexist with hematite only along the hematite-magnetite boundary, but not within the field of stability of hematite proper. This was demonstrated experimentally for Mg-rich biotites (80 weight per cent phlogopite), employing a Cu/Cu₂O buffer, which has a slightly higher oxygen pressure than magnetite/hematite at 650° C.

On surface II, which represents biotites coexisting with magnetites, data determined with a Ni/NiO buffer are shown. The P_{O_2} - T curve for Ni/NiO lies well within the magnetite field.

often figure 12 must be consulted to elucidate specific spatial relations.

The Phlogopite-Annite Join

D. R. Wones

The composition of biotites on the phlogopite-annite join coexisting with (1) hematite + magnetite + sanidine + vapor, (2) magnetite + sanidine + vapor, and (3) olivine + leucite + kalsilite + vapor at fixed temperatures, total pressure, and P_{O_2} is being studied. The results will determine the location of the surfaces I, II, III, and IV of figure 12.

Oxygen buffers are used in all runs. Loss of iron into the platinum crucibles

is minimized by placing a silver capsule within the platinum tube and surrounding it with the same material as the charge.

Compositions of the equilibrium biotites are determined by measuring the indices of refraction and the position of the (060) reflection. Standards were synthesized under a variety of conditions. Some experiments yield small amounts (1 per cent) of metastable sanidine, magnetite, and pyroxene. Figure 14 shows index of refraction and $d(060)$ in Å. U. plotted as a function of composition of biotites synthesized at 600°, 700°, and 800° C, 30,000 psi total pressure, using P_{O_2} of a Ni-NiO buffer. Indices of refraction appear to be functions of P_{O_2} as well as Fe/Mg ratios with effects most pronounced in the iron-rich members.

Phase boundaries were established by forming equilibrium assemblages at a given P_{tot} ($=P_{H_2O}+P_{H_2}$), temperature, and P_{O_2} from a homogeneous biotite as well as from a mixture of $K_2O \cdot 4SiO_2$, SiO_2 , $\gamma-Al_2O_3$, MgO , Fe , and H_2O at different bulk compositions. Runs of different durations demonstrated that 5 days are necessary to achieve equilibrium. In figure 14 it may be noted that biotites of 80 and 90 per cent annite (by weight) react at 800° C to form a biotite of the composition 60 per cent annite + sanidine + magnetite + vapor. Line HH' in figures 12 and 13 is the result of these experiments and defines surface II at the particular sets of T and P_{O_2} used. Corresponding experiments are being carried out using buffers $Cu+Cu_2O$, $Fe_2O_3+Fe_3O_4$, quartz + fayalite + magnetite, Fe_3O_4+FeO , and $FeO+Fe$.

Ferrous-Ferric Biotites

D. R. Wones

Further studies of the substitution of Fe in micas have been made by synthesizing a mica of the composition $K_2O \cdot 6FeO \cdot Fe_2O_3 \cdot 6SiO_2$. This aluminum-free biotite was originally described by Veres, Merenkova, and Ostrovski (1955). Its stability field is quite different from that of the aluminous analog, annite. The following

sequence of mineral assemblages at 30,000 psi total (water) pressure for a series of oxygen pressures was found: (1) P_{O_2} of a hematite + magnetite buffer: 660° C, ferrous-ferric biotite; 670° C, iron-sanidine + hematite + magnetite; 680° C, melt + hematite + magnetite. (2) P_{O_2} of a quartz + magnetite + fayalite buffer: 795° C, ferrous-ferric biotite; 800° C, melt + magnetite. (3) P_{O_2} of a wüstite + magnetite buffer: 680° C, ferrous-ferric biotite; 750° C, melt + fayalite + magnetite. (4) P_{O_2} of an iron + wüstite buffer: 700° C, ferrous-ferric biotite; 750° C, fayalite + melt.

At 850° C, 30,000 psi total (water) pressure, and the P_{O_2} of an iron + wüstite buffer, these biotites recrystallize before melting, forming crystals as large as a millimeter in diameter. Single crystals prepared under these conditions are being used in crystal-structure analysis by G. Donnay and co-workers.

CORDIERITE- H_2O SYSTEM

W. Schreyer and H. S. Yoder, Jr.

Cordierite is often a constituent of extrusive as well as intrusive igneous rocks of a wide range of composition, but the most common occurrence is in metamorphic contact aureole rocks such as spotted slates and hornfelses. The mineral is also encountered in some regionally metamorphosed rocks such as the cordierite-anthophyllite schists, cordierite-sillimanite gneisses, granulites, and charnockites. Knowledge of the stability of cordierite and its polymorphs contributes, therefore, to an understanding of a wide range of rock types.

Cordierite is usually assumed to have the composition $(Mg,Fe)_2Al_3(AlSi_5)O_{18}$, but most analyses of natural cordierites show up to 3 per cent water and minor amounts of alkalis. It was necessary, therefore, to study the system $Mg_2Al_3(AlSi_5)O_{18}-Fe_2Al_3(AlSi_5)O_{18}$ both dry and in the presence of water. The problems concerning its polymorphism, composition, and lower stability limits were in-

vestigated in the magnesium end member first.

Polymorphism

Previous experimental work indicated that at least three forms of $\text{Mg}_2\text{Al}_3(\text{AlSi}_5)\text{O}_{18}$ existed: a high-temperature form synthesized in the dry way, now called indialite ($=\alpha$ -cordierite); a low-temperature form synthesized hydrothermally, called cordierite ($=\beta$ -cordierite); and a metastable form, called μ -cordierite. The inversion between cordierite and indialite was believed to be about 830°C at 1000 bars water pressure (Yoder, 1952) extending to about 600°C at 200 bars water pressure (Iiyama, 1958). These polymorphs were distinguished on the basis of refractive indices. Recent X-ray measurements on natural material confirmed the existence of a high-temperature indialite and a low-temperature cordierite, and demonstrated that there were structural states intermediate between the two forms (Miyashiro, 1957).

The present hydrothermal studies at 2000 and 5000 bars water pressure confirm the existence of intermediate states in the vicinity of the alleged inversion on the basis of indices of refraction. It is not known as yet whether these states are time dependent, mainly because the optical properties and X-ray diffraction properties do not appear to change concomitantly. That is, a change in the refractive indices may take place without a significant change in the X-ray diffraction pattern, and vice versa. It can be said with certainty that a low-temperature form of cordierite identical to that from Albany County, Wyoming, and Guilford, Connecticut, has been synthesized. A re-examination of cordierites previously prepared hydrothermally (Yoder, 1952) indicated they were similar to but not identical with the low-temperature form.

Composition

The shape of the curve representing the beginning of the change from cordierite

to some intermediate state is similar to that of a decomposition curve of a hydrous mineral. Experiments are now under way to determine whether the low-temperature form contains water, and to correlate the water loss of natural cordierites with any changes in optical and X-ray properties.

In addition to the question of the water content, there is also some doubt as to the ratio of the nonvolatile components. The cordierites synthesized in the wet way always exhibit minute inclusions which look like tiny spinel crystals. Assuming that they are spinels, this fact might be attributed to loss of silica to the vapor. Further investigations will be necessary to indicate the true nature of these inclusions, which might be liquid as well. All our runs on materials of the cordierite composition above about 800°C , however, indicated clearly a loss of silica as well as some magnesia to the vapor. At these elevated temperatures, but well below the incongruent melting of cordierite, we obtained minor amounts of spinel (outside the cordierite crystals), sapphirine, and corundum in addition to cordierite. The quenched vapor consisted partly of balls up to 0.5 mm in diameter of a highly siliceous glass with a very low refractive index.

Lower Stability Limits

Magnesium cordierite is known to be stable up to the solidus, where it breaks down to mullite and liquid. Its lower stability limit, however, has only been examined in a cursory way. Both natural and synthetic materials of the magnesium cordierite composition were subjected to a wide range of water pressures and temperatures to fix the lower stability limit of cordierite. Below about 500°C , 2000 bars, and 550°C , 5000 bars, the stable assemblage is chlorite (amesite) + pyrophyllite. In the vicinity of 400°C pyrophyllite gives way to a magnesium-bearing montmorillonite. These magnesian montmorillonites have a wide range of solid solution, and possibly a montmorillonite solid solution

of the cordierite composition itself exists at the lowest temperatures. On the basis of the experiments performed it is believed that cordierite is produced in a sediment of the requisite composition while undergoing metamorphism in the following way. The original sediment probably contains a magnesian montmorillonite solid

semblage. The relation of cordierite to chlorite, pyrophyllite, montmorillonite, and a hypothetical hydrous cordierite, $2\text{MgO} \cdot 2\text{Al}_2\text{O}_3 \cdot 5\text{SiO}_2 \cdot \text{H}_2\text{O}$, is shown in figure 15. The water-deficient region is stippled. In this region cordierite may be stable at temperatures as low as that on the earth's surface.

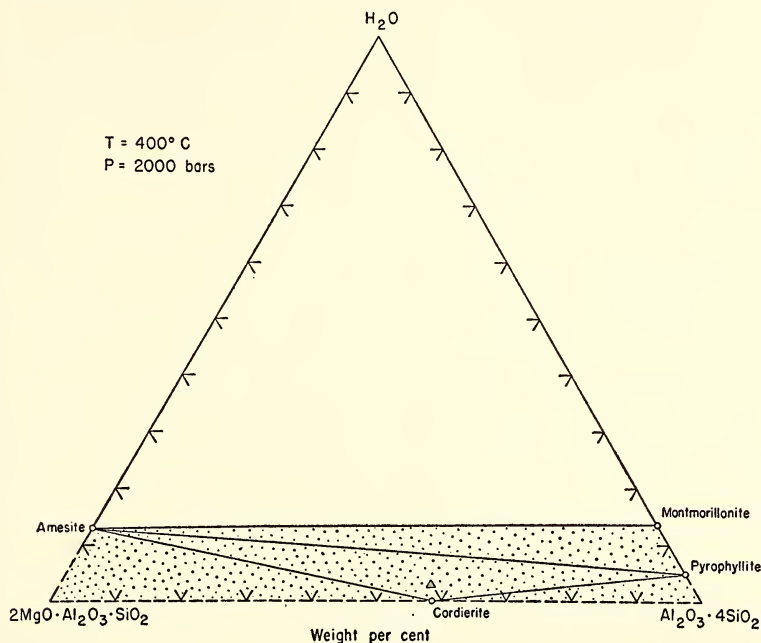


Fig. 15. Assemblages stable at approximately 400° C and 2000 bars in the section $2\text{MgO} \cdot \text{Al}_2\text{O}_3 \cdot \text{SiO}_2 - \text{Al}_2\text{O}_3 \cdot 4\text{SiO}_2 - \text{H}_2\text{O}$. The solid solution of magnesian montmorillonite has been neglected. The triangular point is the hypothetical composition $2\text{MgO} \cdot 2\text{Al}_2\text{O}_3 \cdot 5\text{SiO}_2 \cdot \text{H}_2\text{O}$. The portions of the triangle bordered by dashed lines contain additional phases in $\text{MgO} - \text{Al}_2\text{O}_3 - \text{SiO}_2 - \text{H}_2\text{O}$ not represented in this section. The stippled area is the water-deficient region.

solution and possibly an aluminous serpentinite. The latter inverts to a chlorite and the montmorillonite becomes less magnesium rich with increasing temperature. Then the montmorillonite, essentially free of magnesium, inverts to pyrophyllite. At some higher temperature the chlorite and pyrophyllite react to form cordierite. Such may be the growth sequence leading to a cordierite-bearing spotted slate.

Future studies of cordierite will include investigation of its behavior in the water-deficient region with water contents less than that in the chlorite-pyrophyllite as-

ANHYDROUS ALKALI-FREE CORDIERITES

W. Schreyer and J. F. Schairer

The basic structure of cordierites contains Mg, Al, Si, and O, and it possesses channels which may under appropriate conditions be filled in whole or in part with water or alkalis. In order to ascertain the possibilities of variations in the composition of anhydrous alkali-free cordierites, we are at present re-examining parts of the system $\text{MgO} - \text{Al}_2\text{O}_3 - \text{SiO}_2$. In their study of the system $\text{MgO} - \text{Al}_2\text{O}_3 - \text{SiO}_2$, Rankin and Merwin (1918) indicated that there might be solid solution in

cordierite between the limits $2\text{MgO} \cdot 2\text{Al}_2\text{O}_3 \cdot 5\text{SiO}_2$ and $\text{MgO} \cdot \text{Al}_2\text{O}_3 \cdot 3\text{SiO}_2$. We have prepared a series of melts on the line spinel ($\text{MgO} \cdot \text{Al}_2\text{O}_3$)–silica with 47, 48, 50, 51.35 (2:2:5), 52.5, 53.5, 54.5, 55.87 (1:1:3), 56.5, 58, 60, 65.95, 67.40, 69.84, and 73.24 weight per cent silica. These melts have been crystallized and are being subjected to thermal, optical, and X-ray studies. It is already apparent that there is no variation in the composition of cordierite between 2:2:5 and spinel. The composition 50 per cent SiO_2 when completely crystalline consists of cordierite with an amount of spinel easily detected under the microscope. Cristobalite is present with cordierite and can also easily be detected under the microscope in the completely crystallized compositions with 56.5 per cent silica or more. Studies of compositions between 2:2:5 and 1:1:3 are now in progress. If the composition of cordierite is variable owing to solid solution, crystallization paths of the points lying in the field of cordierite should be curved rather than straight. Nine compositions that should lie in the field of cordierite have been prepared, and a study of their crystallization paths is now in progress.

In order to study the course of crystallization of the cordierite polymorphs, glass of pure 2:2:5 composition was subjected at atmospheric pressure to various temperatures for various lengths of time. No crystallization was observed at 500° and 700° C after 2 months. At 800° C after 22 days the sample showed no sign of devitrification when examined optically but yielded a faint peak in the X-ray pattern. After 58 days the rims of the glass fragments were birefringent and the X-ray pattern was similar to that of quartz, but with the peaks definitely shifted to lower 2θ angles. At 900° C after 1 day the glass had crystallized to the same quartzlike structure, which after longer heating gradually disappeared and gave rise to a cordierite structure. At the temperatures 1000°, 1100°, 1200°, and 1300° C the glass readily

crystallized to cordierite. According to its thermal behavior and optical properties, the metastable quartzlike structure obtained at 800° and 900° C corresponds to the so-called μ -form of cordierite described by Rankin and Merwin (1918). In a recent study Karkhanavala and Hummel (1953) also obtained the μ -form and believed it to have a structure similar to that of β -spodumene, $\text{LiAlSi}_2\text{O}_6$. Our own X-ray studies, however, suggest that its structure is more closely related to that of eucryptite, LiAlSiO_4 . Winkler (1948) has synthesized this mineral, and on the basis of single-crystal studies indicated that it had a high-quartz structure with the Li ions filling a hollow spiral in the lattice. In a study of the lithium metasilicate–spodumene–silica system Roy and Osborn (1949) encountered an apparently similar metastable quartzlike phase, which can take various amounts of Li into solid solution. Since the Mg ion has essentially the same size as the Li ion, it is likely that our silica phase contains Mg in solid solution. It is not certain, however, that its composition is the same as that of cordierite, in other words, that the glass has completely crystallized to this phase.

The X-ray patterns of all the cordierites made in the temperature range between 900° and 1250° C showed a single sharp peak between 29° and 30° 2θ . According to Miyashiro (1957), therefore, they are indialites, the so-called high-temperature form of cordierite. For most of the high-melting compositions examined, runs of 5 days or longer at temperatures between 1420° and 1450° C, however, yielded products exhibiting at least two peaks in this range, in this respect corresponding closely to most of the natural cordierites. Either type of pattern or an intermediate stage may result from a run of less than 5 days at this temperature. The one- and multi-peak phases do not differ in refractive indices. The effect of slow cooling and of quenching at various rates from a series of temperatures is now under investiga-

tion. Cordierites crystallized from the lower-melting compositions in the cordierite stability field may represent the multi-peak form at temperatures as low as 1350° C.

ALKALI AMPHIBOLES

W. G. Ernst

The most important alkali amphiboles belong to the glaucophane-riebeckite series,

been reported. Extensive solid solution exists among the other three end members.

Glaucophane and riebeckite are found principally in low-grade metamorphic rocks; some iron-rich riebeckites occur in alkalic igneous bodies. The presence of sodic amphiboles in rocks of diverse composition has led to much speculation about their mode of origin. Laboratory investigation has been undertaken in an attempt

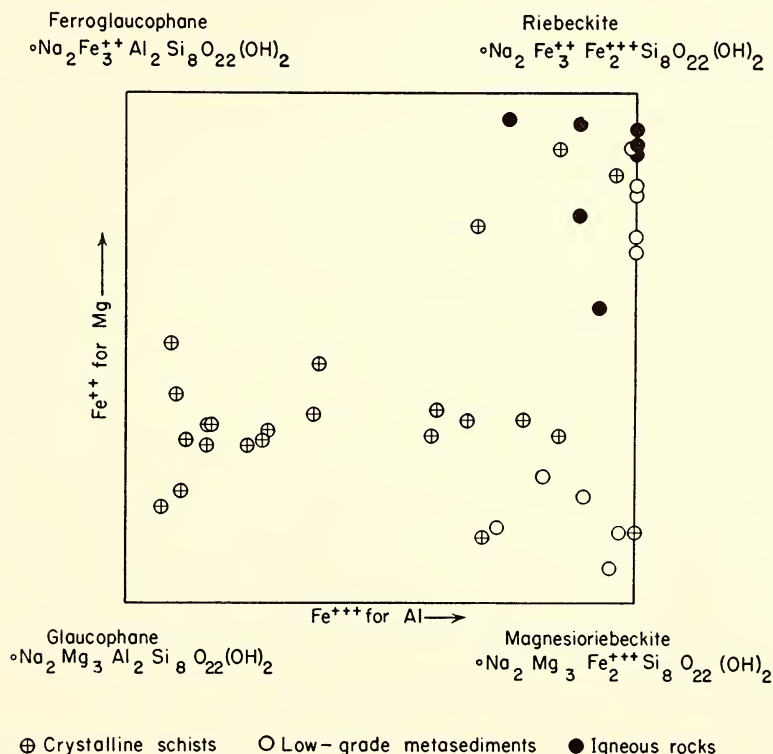


Fig. 16. Chemical variation in natural members of the glaucophane-riebeckite series.

which has four end members: glaucophane, $^{\circ}\text{Na}_2\text{Mg}_3\text{Al}_2\text{Si}_8\text{O}_{22}(\text{OH})_2$; ferroglaucophane, $^{\circ}\text{Na}_2\text{Fe}_3^{++}\text{Al}_2\text{Si}_8\text{O}_{22}(\text{OH})_2$; magnesioriebeckite, $^{\circ}\text{Na}_2\text{Mg}_3\text{Fe}_2^{+++}\text{Si}_8\text{O}_{22}(\text{OH})_2$; and riebeckite, $^{\circ}\text{Na}_2\text{Fe}_3^{++}\text{Fe}_2^{+++}\text{Si}_8\text{O}_{22}(\text{OH})_2$. (The symbol $^{\circ}$ represents a vacant position in the structure.)

Chemical analyses of natural glaucophanes and riebeckites are plotted in figure 16. No amphibole closely approaching the composition of ferroglaucophane has

to evaluate the physical and chemical parameters that govern the stability of these amphiboles.

Magnesioriebeckite

X-ray diffractometer patterns of synthetic magnesioriebeckite crystallized over a wide P_{O_2} - P_{vapor} - T range are mutually indistinguishable, and are similar to that of natural magnesioriebeckite from Cochabamba, Bolivia (USNM 4980). Calcu-

lated lattice parameters for the synthetic amphibole are somewhat different from those determined by Whittaker (1949) for another sample of the Cochabamba material (see table 4).

TABLE 4. Unit Cell Dimensions of Synthetic and Natural Magnesioriebeckite

Synthetic Magnesioriebeckite	Natural Magnesioriebeckite *
$a = 10.04 \pm 0.01 \text{ \AA}$	$a = 9.89 \text{ \AA}$
$b = 18.02 \pm 0.02 \text{ \AA}$	$b = 17.95 \text{ \AA}$
$c = 5.28 \pm 0.01 \text{ \AA}$	$c = 5.31 \text{ \AA}$
$B = 72^\circ 01' \pm 03'$	$B = 72\frac{1}{2}^\circ$

* E. J. W. Whittaker (1949).

acterizes magnesioriebeckite grown at high partial pressure of oxygen; at fixed partial oxygen pressure, magnesioriebeckite crystallized at high temperature has a low refractive index. Equilibrium has been demonstrated by cycling synthetic magnesioriebeckite at both higher and lower P_{O_2} ; refractive indices are independent of starting material and duration of run. Refractive indices are assumed to be a measure of the oxidation state of the iron in magnesioriebeckite: the lower the index of refraction, the smaller the Fe_2O_3/FeO ratio. Electrostatic neutrality is probably maintained (1) through replacement of oxygen by hydroxyl, (2) through partial occu-

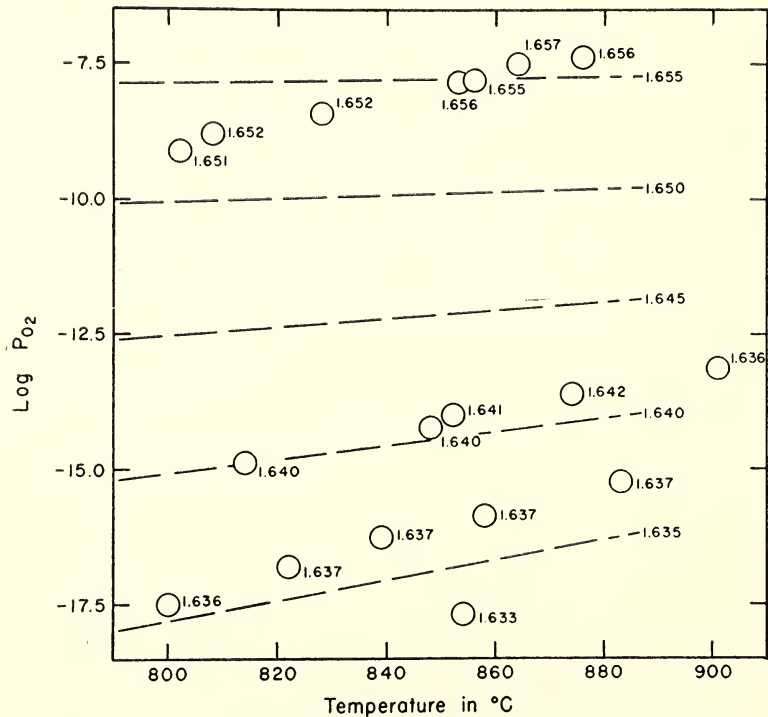


Fig. 17. Variation in N_x of magnesioriebeckite with P_{O_2} and T . The apparently anomalous refractive index of the amphibole crystallized at 901°C results from the fact that it formed within the melting interval of magnesioriebeckite (described last year) and is more magnesian than the phase stable below the magnesioriebeckite solidus surface.

The variation in optical properties of synthetic magnesioriebeckite with P_{O_2} and T is illustrated in figure 17. At a given temperature, high index of refraction char-

acterizes magnesioriebeckite grown at high partial pressure of oxygen; at fixed partial oxygen pressure, magnesioriebeckite crystallized at high temperature has a low refractive index. Equilibrium has been demonstrated by cycling synthetic magnesioriebeckite at both higher and lower P_{O_2} ; refractive indices are independent of starting material and duration of run. Refractive indices are assumed to be a measure of the oxidation state of the iron in magnesioriebeckite: the lower the index of refraction, the smaller the Fe_2O_3/FeO ratio. Electrostatic neutrality is probably maintained (1) through replacement of oxygen by hydroxyl, (2) through partial occu-

pancy of the vacant position by cations (H_3O^+ ?), or (3) through minor solid solution with grunerite or arfvedsonite.

The influence of partial oxygen pressure

on the stability field of magnesioriebeckite is shown in figure 18. At a specified total pressure, increased P_{O_2} elevates the temperature at which magnesioriebeckite breaks down. All condensed phases stable above the amphibole field contain both MgO and

gen pressure declines, causing all magnesium-bearing phases to become enriched in ferrous iron. The orthopyroxene stable adjacent to the magnesioriebeckite field changes composition from En_{98} with a hematite-magnetite buffer to En_{79} with a

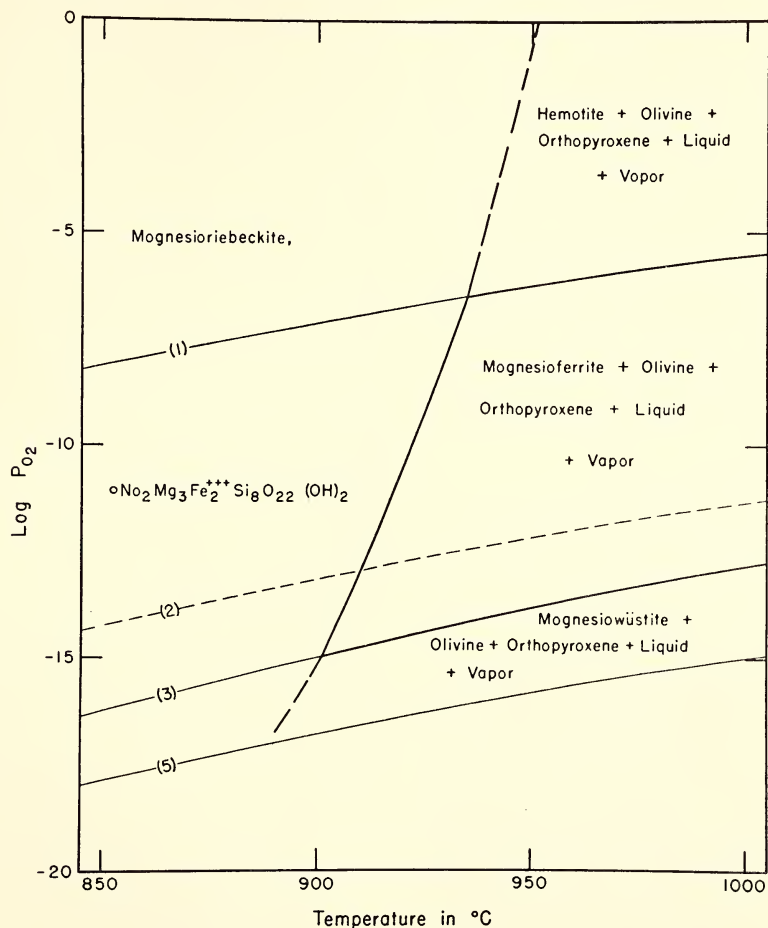


Fig. 18. P_{O_2} - T diagram for the composition $Na_2O \cdot 3MgO \cdot Fe_2O_3 \cdot 8SiO_2$ with excess water at 2000 bars vapor pressure. Curves (1), (2), (3), and (5) are defined by the oxygen buffers hematite-magnetite, magnetite + silica-fayalite, magnetite-wüstite, and wüstite-iron, respectively.

FeO, with the exception of hematite and possibly acmite. The ratio MgO/FeO among coexistent phases is highest in the lowest-melting crystalline phase, lower in the more refractory minerals, and lowest in the liquid. At fixed temperature and total pressure, the MgO/FeO ratio of the bulk composition decreases as the partial oxy-

magnetite+silica-fayalite buffer, and to En_{73} with a magnetite-wüstite buffer; coexistent olivine over the same P_{O_2} interval changes from Fo_{95} to Fo_{73} and to Fo_{65} . Magnesioferrite decreases in amount (as a greater portion of the ferric iron is reduced) and approaches the composition Fe_3O_4 with a magnetite-wüstite buffer.

The effect of the partial pressure of oxygen on the composition and stability relations of minerals encountered in this system illustrates the petrologic importance of this variable.

An intermediate member of the magnesioriebeckite-riebeckite series has been found as an authigenic mineral in the Green River shale of the Colorado Plateau, indicating that the magnesioriebeckite stability field extends to low temperatures and pressures. The rarity of this mineral is in part due to the fact that few rocks contain Na_2O in excess of Al_2O_3 . However, magnesioriebeckite has been found in a few crystalline schists whose bulk compositions show no excess of soda over alumina. In this case, the presence of sodic amphibole suggests special physical conditions; the nature of these conditions will be discussed in the next section.

Glaucophane

The preliminary P - T stability field of $^\circ\text{Na}_2\text{Mg}_3\text{Al}_2\text{Si}_5\text{O}_{22}(\text{OH})_2$ up to 2000 bars vapor pressure is presented in figure 19. The slope of the breakdown curve of this amphibole is unusually steep.

Forsterite, enstatite, albite, glaucophane, liquid, and vapor coexist at an invariant point at 867°C and 1500 bars. With pressures greater than 1500 bars, glaucophane melts incongruently to forsterite, enstatite, liquid, and vapor. The high-temperature assemblage adjoining the glaucophane field at pressures lower than 1500 bars consists of forsterite, enstatite, albite, and vapor.

At 966°C and 500 bars, the melting curve of albite and the incongruent melting curve of enstatite intersect. Below 500 bars, the liquidus curve for enstatite coincides with solidus curves for enstatite and albite; above 500 bars, the liquidus curve for albite coincides with solidus curves for albite and enstatite. The locus of the intersection is a point on a univariant curve along which five phases (forsterite, enstatite, albite, liquid, and vapor) are stable. The two liquidus (and solidus) surfaces

intersect for a range of bulk compositions.

Results of the experimental investigation indicate that glaucophane is not itself a high-pressure mineral. This fact is of considerable petrologic significance since many geologists consider glaucophane the characteristic mineral of a low-temperature, high-pressure metamorphic facies. Their conclusion is based on the evidence that (1) glaucophane schists are more dense than parent sediments and lavas, and typical metamorphic rocks; (2) many glaucophane schists are chemically equivalent to green schists and amphibolites; (3) glaucophane schists are developed on a regional scale in some areas; and (4) jadeite, experimentally demonstrated to be a high-pressure mineral, has been found in certain glaucophane schists. Other workers have cited the unusual bulk compositions of many glaucophane schists, local development of glaucophane schists, often adjacent serpentinites, and the intimate association of glaucophane schists with green schists and amphibolites as indication that glaucophane schists owe their production to peculiar chemical conditions.

In an attempt to integrate field and laboratory data, glaucophane schist localities of the California coast ranges were visited during July and August 1957. Irregularly distributed schist bodies have developed locally in the severely folded and faulted Franciscan formation, a series of arkosic subgraywackes with intercalated argillaceous cherts, basaltic and spilitic lavas, and serpentine intrusives; small masses of eclogite are present rarely.

The Tres Pinos Creek area (San Benito quadrangle) was examined in some detail. Here glaucophane- and riebeckite-bearing schists have developed from graywackes, cherts, and basic extrusives with no apparent relation to serpentinite. Gradational contacts between sheared Franciscan formation and glaucophane schist indicate that the initial stage of metamorphism involved granulation and the production of lawsonite, minor glaucophane, and stilp-

nomelane; in highly recrystallized schists, lawsonite has been replaced by aggregates of epidote or clinozoisite. Except for the relative proportions, metagraywackes and metabasalts display similar mineralogy, consisting of glaucophane-crossite+lawsonite or clinozoisite-epidote+stilpnomelane;

these glaucophane schists probably formed at low temperatures. The restriction of the metamorphic rocks to zones of shearing may be an indication that unusual pressures were required for the formation of these alkali amphibole-bearing rocks and/or that solutions migrating along fa-

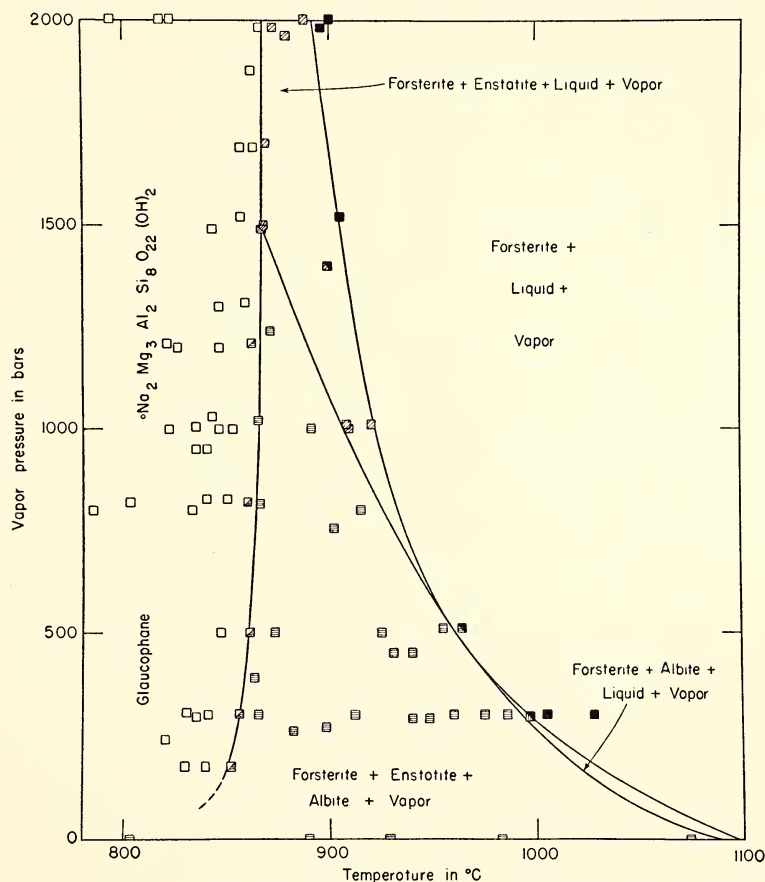


Fig. 19. Preliminary $P_{\text{vapor}}-T$ diagram for the composition $\text{Na}_2\text{O} \cdot 3\text{MgO} \cdot \text{Al}_2\text{O}_3 \cdot 8\text{SiO}_2$ with excess water.

lane+muscovite+quartz (+garnet) + ores. Metacherts contain crossite-riebeckite +sodic pyroxene+stilpnomelane+quartz (+garnet?) +ores. The different mineralogy of the metacherts is due to the low CaO and Al_2O_3 content. Albite is absent in metamorphic rocks of the area investigated (except as a vein mineral). Rapid lateral gradation into unmetamorphosed Franciscan formation suggests that

vorable channelways fluxed the relatively sluggish reaction involving conversion of granulated sediments, flows, and cherts to a stable low-grade metamorphic assemblage.

Returning to the general problem, it may be observed that metamorphic rocks of various compositions generally contain both amphibole and plagioclase. The partition of Na_2O and CaO among these and

other phases depends on the physical and chemical conditions accompanying the metamorphism; commonly sodium is concentrated in feldspar while amphibole and other ferromagnesian minerals contain the calcium. Where special bulk compositions are deficient in CaO, as in the experimental investigation, glaucophane can exist over a wide *P-T* range. Rock compositions with soda in excess of alumina would favor the production of intermediate members of the glaucophane-riebeckite series, since they contain more Na₂O than Al₂O₃. The instability of sodic plagioclase in rocks of normal chemical composition at high pressure would also promote the formation of sodic amphibole or sodic pyroxene, or both.

In conclusion it may be stated that, although some glaucophane-bearing rocks are formed under common metamorphic temperatures and pressures in response to hypersodic chemical conditions, others are rocks of normal bulk compositions that have been subjected to relatively high total pressures.

Riebeckite

Investigation of ${}^{\circ}\text{Na}_2\text{Fe}_3^{++}\text{Fe}_2^{+++}\text{Si}_8\text{O}_{22}(\text{OH})_2$ is under way. To pressures in excess of 2000 bars, riebeckite breaks down to fayalite, magnetite, quartz, acmite, and vapor, using a magnetite + silica-fayalite buffer. Preliminary data at 2000 bars indicate that the stability limit of riebeckite lies between 600° and 700° C.

COMPOSITION OF A PSEUDOLEUCITE FROM THE BEARPAW MOUNTAINS, MONTANA

E. G. Zies and F. Chayes

The pseudoleucites rank high among the familiar but little-understood mineral associations characteristic of alkaline rocks. The mineral leucite occurs primarily in volcanic rocks; it is found occasionally in dike rocks or very shallow intrusives clearly associated with volcanics, but has never been described from a truly plutonic environment. From a number of alkaline areas mineral aggregates are known

which exhibit—sometimes poorly, sometimes with remarkable sharpness—the crystal form of leucite, but contain none of that mineral. These pseudoleucites are usually mixtures of sanidine or orthoclase with one or more of the feldspathoids or zeolites. Nepheline is perhaps the commonest, but sodalite, cancrinite, and analcite are all well known.

The leucite-like shape was long ago taken as an indication that at some stage of their history these aggregates had in fact been leucite. As far as is known, however, natural leucite is always poor in Na, a constituent ordinarily abundant in pseudoleucite. Conversion of an initially Na-poor leucite into an Na-rich aggregate of nepheline and orthoclase was regarded by Bowen as an expectable consequence of crystal fractionation from melts of appropriate composition. He made it the basis of an ingenious theory about the origin of alkaline rocks and their relation to biotite granite.

Firm quantitative information about the chemical and mineralogical composition of pseudoleucites is still very scarce. The pseudoleucite in a porphyritic tinguaita from the Bearpaw Mountains, Montana, seemed to afford an ideal opportunity for combined chemical and modal analysis. It was collected by one of us, from a dike that outcrops along the fire road to Elk Peak, during a brief visit to the area under the guidance of R. Schmidt, of the U. S. Geological Survey.

Our specimen contains numerous white subangular nodules, vaguely suggestive of leucite in outline, consisting of intergrowths and mixtures of nepheline and sanidine together with a little aegerine and an occasional grain of other minerals. No analcime or sodalite has been noted; the nepheline is virtually unaltered; and the occasional fibrous alteration (sericite or cancrinite) on sanidine is so scarce that it has not been possible to identify it properly.

The white patches are mostly between

$\frac{1}{2}$ and 1 cm in maximum dimension, but they do not liberate readily from the matrix of the rock until crushed to a size at which hand sorting is impracticable. Rock fragments were therefore crushed to pass a no. 7 cloth, and a concentrate was obtained by electromagnet from the fraction retained on a no. 9 cloth. The bulk analysis of this concentrate and the analyses of portions of it soluble and insoluble in boiling 1:5 HCl are shown in table 5.

TABLE 5. Chemical Analyses of a Pseudoleucite from Elk Peak, Bearpaw Mountains, Montana

	Entire Sample	Acid-Soluble Portion	Acid-Insoluble Portion
SiO ₂	59.62	41.0	63.96
Al ₂ O ₃	20.69	33.1	17.95
Fe ₂ O ₃	1.39	2.6	0.99
TiO ₂	0.07	0.1	0.08
CaO	0.10		
BaO	0.29		0.45
Na ₂ O	3.39	15.1	0.66
K ₂ O	14.43	8.1	15.91
H ₂ O ⁺	0.20		
H ₂ O ⁻	0.02		
SO ₃	0.02		

Table 6 gives the results of modal analyses of two types; columns A and B are the average modes of six stained and five unstained thin sections cut from chips, parts of which were used to obtain the magnetic concentrate. Column C is the average of modes made on five microsamples of the ground, sized, and purified concentrate actually used for the chemical analysis.

The purification procedure is considerably less extreme, particularly as regards washing and desliming, than that often resorted to in sample preparation of this type. It has nevertheless led to a shift in the ratio of nepheline to feldspar so extreme that comparison of chemical and modal results would have been unintelligible or misleading if carried through directly from bulk analysis to thin-section modes.

Materials reprecipitated from the acid solution amounted to 20.1 per cent of the initial sample, and from SiO₂, Na₂O, and K₂O entries in table 5 the calculated weight per cent of the acid-soluble portion is 18.8 per cent. The sharp discrepancy between either of these values and the nepheline entry (or the sum nepheline + acmite) in column A of table 6 led to the analyses that yielded column B. The agreement between these two columns stimulated much speculation about the "actual" composition of the minerals, spec-

TABLE 6. Average Modes of a Pseudoleucite from Elk Peak, Bearpaw Mountains, Montana

	A	B	C
Sanidine	66.9	65.4	83.1
Nepheline	29.0	30.7	15.0
Acmite	3.3	2.8	} 1.9
Others	0.8	1.0	
A. Mean of 112 aggregates in 6 stained thin sections.			
B. Mean of 111 aggregates in 5 unstained thin sections.			
C. Mean of 5 microsamples from final powder used for chemical analysis.			

ulation that was brought to an abrupt halt by the analysis given in column C, which shows that the modal content of nepheline has indeed been drastically reduced by the sample preparation. We conclude that the nepheline and sanidine of the pseudoleucite have essentially the compositions shown in columns 2 and 3 of table 5, but that the amounts of these two minerals actually present in the pseudoleucite are better estimated by columns A and B of table 6. It is interesting to note that a nepheline rather rich in K₂O but in almost exact balance with regard to SiO₂ occurs, in intimate intergrowth, and apparently in equilibrium, with a sanidine unusually poor in Na₂O. A coarse-grained nepheline syenite consisting largely of nepheline and feldspar with compositions like those in our pseudoleucite has been described from Assynt by Tilley. He has also described a Brazilian pseudoleucite

which must be very similar, but ours appears to be the first of which actual analyses are available for both phases.

The details of this study will be published separately. In much petrographic work of this general type the only quantitative data would be the bulk analysis of the concentrate. More rarely, the thin-section mode would also be available. The bulk analysis alone would lead to one description. The combination of thin-section mode and bulk analysis would lead to a very different one, and from this combination the petrologist might be encouraged to suppose that the nepheline observed under the microscope was extraordinarily siliceous. The analysis of the acid-soluble fraction dispels this fantasy but leaves unresolved a glaring discrepancy between petrographic and chemical estimates of the mode. The discrepancy might be explained by supposing that the thin-section modes are absurdly faulty, but comparison of column C with column A or B of table 6 indicates that the real difficulty is in sample preparation.

FELDSPAR INVESTIGATIONS

P. M. Orville

As part of a continuing program of applying laboratory data to petrologic problems, a study is being made of coexisting plagioclase and alkali feldspars from pegmatites in the southern Black Hills, South Dakota; the Spruce Pine district, North Carolina; and New Hampshire. The perthitic texture of most alkali feldspars from pegmatites offers convincing evidence that movement of ions within the crystal did not cease at the moment of final crystallization. The scale of perthite exsolution textures suggests that the limits within which unmixing of feldspar phases and migration of alkali ions have taken place is of the order of a few millimeters. On this basis it seems reasonable to assume that the *central portion* of a perthite crystal many inches in size has been affected only by an unmixing process taking

place within its own volume and has neither gained nor lost material with respect to its surroundings.

If the assumptions can be made that contiguous plagioclase and perthite crystals have crystallized in equilibrium with one another and that their compositions have not changed subsequent to the time of crystallization as single phases, then the bulk compositions of the two feldspars can be considered to represent the two end points of a tie line at the crystallization temperature within the solidus region of the ternary feldspar system. The orientation of the tie lines and the position of the end points will change as functions of temperature and, to a lesser extent, pressure. Previous reports (Year Book 56, p. 206; 55, p. 190) have indicated the progress made in determining phase relations within the synthetic ternary feldspar system. More data are needed on the position of the tie lines in the subsolidus region before the composition of coexisting natural feldspars can be used as a geothermometer, but it should be possible, on the basis of data now at hand, to obtain an idea of the relative changes in temperature during the formation of successive zones of a zoned pegmatite at an assumed constant pressure.

The composition of ten pairs of contiguous plagioclase and microcline perthite crystals from pegmatites in the Spruce Pine district, North Carolina, has been plotted in figure 20. Ab and Or contents are based on flame photometer determinations of Na and K. The An content of the contiguous plagioclase was determined from the refractive index of a fused sample. The bulk An content of perthite is based on semiquantitative determinations of Ca by the emission spectrograph. The orientations of the tie lines and the position of the end points are consistent with data for the ternary feldspar system and indicate final crystallization at relatively low temperature or high pressure.

The natural perthites, for most pur-

poses, can be considered a ternary system in which Ab and Or are major constituents and An a minor constituent, generally present in amounts less than 2 per cent by weight. Ba and Rb may also be present as minor constituents. There is no single technique except chemical analysis by which the ternary components can be directly determined with adequate precision. Flame photometer determination of K

X is the angular distance in degrees and Y is the composition in terms of weight per cent Or. The corresponding equation for Fe K α radiation is $Y = 167.21 - 73.69X$. This curve has an advantage over determinative curves previously proposed in that a single internal standard which is easily obtainable in pure form has been used over the entire range, and composition of the feldspar is plotted directly

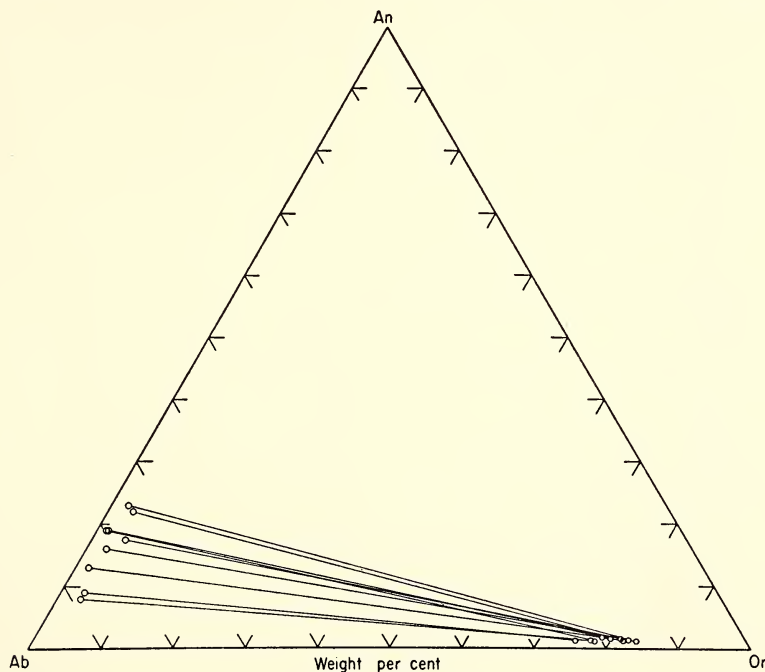


Fig. 20. Plot of contiguous feldspar pairs (plagioclase and microcline perthite) from pegmatites in Spruce Pine district, North Carolina.

and Na permits an estimate of composition in terms of Ab and Or content, and the $(\bar{2}01)$ X-ray technique of Bowen and Tuttle gives a composition in terms of the Or content.

Figure 21 is a newly established determinative curve for the synthetic alkali feldspar series in which the difference in 2θ for Cu K α radiation between the $(\bar{2}01)$ peak of alkali feldspar and the (101) peak of KBrO_3 is plotted directly against the composition in terms of weight per cent Or. The curve is a straight line, the equation of which is $Y = 166.39 - 92.31X$, where

against $\Delta 2\theta$ values. In the original X-ray determinative curve presented by Bowen and Tuttle, composition was plotted against spacings of the $(\bar{2}01)$ plane in the feldspar crystals. An olivine powder with a peak at 2θ of approximately 23° was taken as an internal standard, and the absolute position of the olivine peak was determined by calibration against a quartz peak having a 2θ value of 20.85° . The curve of Chayes and Robbins (Year Book 53, p. 135) was determined only for the Or-rich end of the series between Or_{100} and Or_{60} . The internal standard for this

curve was an Amelia albite with ($\bar{2}01$) peak at $2\theta = 22.06^\circ$.

KBrO₃ seems well suited for use as an internal standard. It is a standard chemical reagent and obtainable in very pure form. The position of the (101) peak ($2\theta = 20.205^\circ \pm 0.010^\circ$) does not differ appreciably between different lots of reagent grade material. The salt is only slightly soluble in water and is much less hygroscopic than most alkali halide salts. Smear mounts prepared with KBrO₃ as an in-

precipably from Ab to An within the synthetic plagioclase series. Therefore, it appears likely that, within the ternary feldspar system, the position of the ($\bar{2}01$) peak is primarily a function of the weight per cent Or.

The curve of figure 21 can be used directly for estimating the Or content of homogeneous natural sanidines or of any natural alkali feldspar that has been homogenized and inverted to the high-temperature monoclinic form. This has been

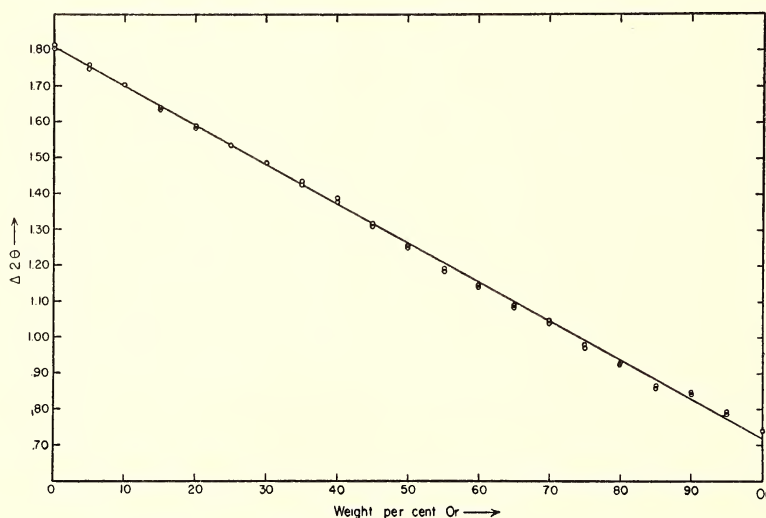


Fig. 21. Difference in 2θ between ($\bar{2}01$) peak of synthetic alkali feldspar and (101) peak of KBrO₃ for Cu K α radiation plotted against composition.

ternal standard have been exposed to the atmosphere for periods of 3 months or more without noticeable change in the position or intensity of the KBrO₃ peaks. The position of the (101) peak is not appreciably affected by a variation in room temperature of 20°C .

The synthetic alkali feldspars on which the curve is based were crystallized from glasses prepared by J. F. Schairer. Crystallization was carried out hydrothermally in sealed platinum tubes at a temperature of 800°C and an H₂O pressure of 1000 bars for periods of 5 to 7 days. A longer crystallization period does not produce significant changes in the ($\bar{2}01$) spacing. The ($\bar{2}01$) spacing does not change ap-

precipably from Ab to An within the synthetic plagioclase series. Therefore, it appears likely that, within the ternary feldspar system, the position of the ($\bar{2}01$) peak is primarily a function of the weight per cent Or.

The curve of figure 21 can be used directly for estimating the Or content of homogeneous natural sanidines or of any natural alkali feldspar that has been homogenized and inverted to the high-temperature monoclinic form. This has been done for a number of coarsely perthitic microclines by heating the finely ground material in sealed platinum tubes at 800°C with 1000 bars H₂O pressure for 25 days.

In table 7 a comparison is made between bulk Or compositions determined by the ($\bar{2}01$) X-ray method and by the flame photometer. The samples are microcline perthites from pegmatites, the first 7 from Spruce Pine, North Carolina, the last 7 from the Black Hills, South Dakota.

The possibility that this curve could also be used to estimate the composition of triclinic alkali feldspars has been investigated. Dry heat treatment of most perthitic microcline at 1050°C for 48

hours results in complete homogenization of the feldspar without inverting it to monoclinic form, although the triclinicity as measured by the difference between the (131) and ($\bar{1}$ 31) peaks decreases somewhat. (Many laboratories lacking hydrothermal apparatus have high-temperature ovens that could be used for the dry homogenization of perthites.) Preliminary results based on X-ray determination of 28 microcline perthites from South Dakota and North Carolina in the composition ranges 70 to 96 weight per cent Or

TABLE 7. Weight Per Cent Or

Sample	($\bar{2}$ 01) X-Ray	Flame Photometer
11-57	73.4	74.6
25-57	77.7	76.4
27-57	78.0	77.9
34-57	79.2	80.4
45-57	82.7	82.3
63-57	81.0	79.3
68-57	76.5	76.9
139-57	73.4	72.8
145-57	89.9	89.0
148-57	90.6	88.4
180-57	84.4	82.4
185-57	85.5	83.8
193-57	87.4	85.4
114-57	76.1	75.1

indicate that samples homogenized in the dry way at 1050° C give Or percentages slightly lower than the same samples homogenized and inverted hydrothermally at 800° C. The average difference between the two values of $\Delta 2\theta$ for the triclinic and monoclinic form of each sample is 0.015°, which is equivalent to 1.5 weight per cent Or.

SPINELS

SpinelS constitute one of the most interesting groups of minerals. They show widespread occurrences as accessory constituents and a few occurrences of great economic importance. Synthetic forms are widely used for many industrial purposes. All spinels consist essentially of a single structural framework of oxygens which

accommodates a great variety of bi- and trivalent cations with the general formula $R^{2+}R^{3+}_2O_4$. Geologically the most important iron-bearing members are magnetite, $FeFe_2O_4$; hercynite, $FeAl_2O_4$; and Fe-chromite, $FeCr_2O_4$.

Buddington and co-workers have demonstrated the geologic significance of solid solution and exsolution phenomena in natural ilmeno-magnetites. For experimental reasons, laboratory investigations of the phase relations in the spinel group have been restricted to high temperatures and atmospheric pressure. Yet elucidation of the subsolidus behavior of iron-rich spinels at moderate temperatures is highly desirable since members of the magnetite group belong to the most common of accessory minerals. Hence the important magnetite-hercynite system ($FeO \cdot Fe_2O_3$ - $FeO \cdot Al_2O_3$) was chosen for investigation.

Magnetite-Hercynite Relations

A. C. Turnock and H. P. Eugster

Atlas and Sumida (1958) report complete solid solution above 1000° C. Yet hercynite is known to have exsolved from many magnetites formed at high temperatures, often in conjunction with exsolution of ilmenite. It was decided, therefore, to investigate the phase relations below 1000° C. The partial pressure of oxygen was controlled by using a quartz+magnetite+fayalite buffer (see Eugster, 1957). The P_{O_2} - T curve for this assemblage lies at all temperatures within the field of stability of magnetite. Complete solid solubility was found above 870° \pm 20° C at a total pressure of 2000 bars. The cell dimensions of the series, synthesized at 800° C and 2000 bars (900° C, 1000 bars for Mt 50 Hc 50), decrease from magnetite $d = 8.393 \pm 0.002$ Å.U. to hercynite 8.149 ± 0.003 . The curve interplanar spacing vs. composition is not a straight line but shows a slightly convex upward curvature on a mole per cent scale, according to $d = 8.393 - 0.00194X - 0.5 \times 10^{-5}X^2$, where X is mole per cent hercynite. This agrees

well with the results of Atlas and Sumida (1958) from experiments done at low pressures in a helium atmosphere.

Preliminary work indicates that at 2000 bars the top of the solvus lies at $870^{\circ} \pm 20^{\circ}$ C, and that the solvus itself is bounded by the following two-phase assemblages (in weight per cent):

800° C	Mt 76 Hc 24—Mt 25 Hc 75
700°	Mt 85 Hc 15—Mt 14 Hc 86
600°	Mt 89 Hc 11—Mt 10 Hc 90
500°	Mt 92 Hc 8—Mt 8 Hc 92

It appears that hercynite is not stable at higher partial pressures of oxygen, such as that of a magnetite-hematite buffer. The hercynite composition is represented by the assemblage magnetite_{ss}+corundum_{ss}. This work will be useful in the interpretation of rocks containing magnetites formed in an aluminous environment, such as magnetites coexisting with hercynite, corundum, and sillimanite.

Chester Emery Deposits

D. R. Wones

The emery deposits at Chester, Massachusetts, have been a famous mineral-collecting locality for many years. In spite of the widespread interest in the mineralogy of the area, intensive geological and petrological studies have not been made. The current efforts have been undertaken to outline the geologic environment and major mineral assemblages of these deposits.

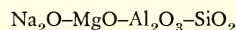
The emery deposits occur as veinlets of corundum-magnetite-hematite rock within a quartz-free mica-chlorite schist. The schist contains accessory tourmaline and magnetite with corundum and zoisite. The chlorite is the variety amesite, as determined by X-ray and optical analysis. The mica is either paragonite or muscovite, depending on location, but no rock has yet been found containing both minerals. Cross-cutting the schist are veins of margarite, chlorite, and diaspore.

The emery-bearing schist is wholly contained within the Chester amphibolite,

near the east border of the amphibolite body. The amphibolite varies from an epidote-amphibole rock to an epidote-oligoclase-amphibole rock. In places the amphibolite and emery are transected by a talc-serpentine rock.

Metasomatic origins have been proposed for emery deposits. X-ray and optical properties of the minerals, many of which are members of solid solutions, do not vary throughout the Chester deposits, indicating homogeneous compositions. Therefore, either metasomatic activity was not a major factor or compositional differences have been erased by subsequent metamorphism.

THE QUATERNARY SYSTEM



J. F. Schairer and H. S. Yoder, Jr.

Two years ago we began a study of this important quaternary system for which phase-equilibrium data were almost completely lacking. Substantial progress was made during the first year of study, and last year (Year Book 56, pp. 217-222, figs. 50-53) we presented phase-equilibrium diagrams for the joins albite-cordierite-silica, albite-forsterite-cordierite, and albite-magnesium metasilicate-cordierite and a diagram showing the relation of univariant lines to ternary invariant points in limiting systems and to seven of the quaternary invariant points. We showed that in the two large volumes albite-corundum-spinel-silica and albite-forsterite-spinel-silica the residual liquid during crystallization proceeds toward a similar goal, a soda-granite.

During the past year we have vigorously pursued the study of the quaternary system, expanding the portion under investigation to include compositions rich in nepheline which lie in the volume nepheline-forsterite-spinel-silica. At this time we present the phase-equilibrium diagram for the system nepheline-spinel-silica as figure 22. Open circles represent the compositions of compounds, and black dots

the compositions studied. This is a ternary system within the quaternary system $\text{Na}_2\text{O}-\text{MgO}-\text{Al}_2\text{O}_3-\text{SiO}_2$ except for those compositions that crystallize mullite ($3\text{Al}_2\text{O}_3 \cdot 2\text{SiO}_2$) at some temperature during their crystallization. Even these become completely ternary in their behavior

angle albite-spinel-cordierite become completely crystalline at the temperature of the ternary reaction point *K*, and the last liquid has the composition *K*; those in the triangle albite-cordierite-silica become completely crystalline at the temperature of *I*, and the last liquid has the composi-

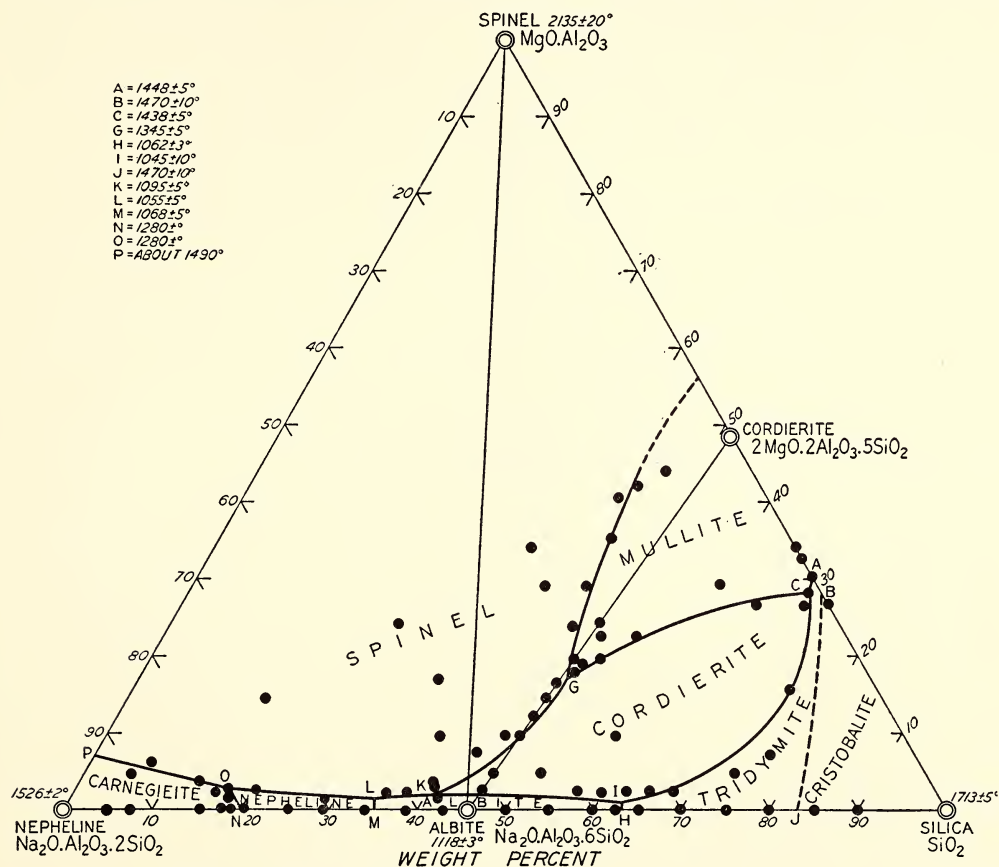


Fig. 22. Phase-equilibrium diagram of the system nepheline-spinel-silica.

at lower temperatures when mullite has disappeared by reaction with liquid.

The portion albite-cordierite-silica was shown in more detail in last year's report (Year Book 56, p. 219, fig. 50), and binary and ternary points are similarly lettered in figure 22. All compositions in the triangle nepheline-spinel-albite become completely crystalline at the temperature of the ternary eutectic *L*, and the last liquid has the composition *L*; those in the tri-

angle albite-spinel-cordierite become completely crystalline at the temperature of the ternary reaction point *K*, and the last liquid has the composition *K*; those in the triangle albite-cordierite-silica become completely crystalline at the temperature of *I*, and the last liquid has the composi-

sidual liquid were separated from early-formed crystals, it would give a nepheline-bearing product of crystallization.

SYSTEMS WITH ROCK-FORMING OLIVINES, PYROXENES, AND FELDSPARS

J. F. Schairer and N. Morimoto

The mutual melting relations among these three important groups of minerals are of primary importance in unraveling the crystallization of many igneous rocks, particularly the basalts. Last year Yoder

olivines, pyroxenes, and feldspars. The groundwork for this study was laid when Bowen (1914) studied the system forsterite–diopside–silica at temperatures where a liquid phase was present. This system depicts the relations of pyroxenes between enstatite ($\text{MgO} \cdot \text{SiO}_2$) and diopside ($\text{CaO} \cdot \text{MgO} \cdot 2\text{SiO}_2$) to the magnesian olivine forsterite ($2\text{MgO} \cdot \text{SiO}_2$) and to silica. Recently Boyd and Schairer (Year Book 56, pp. 223–225) expanded this study of Bowen's to include the subsolidus rela-

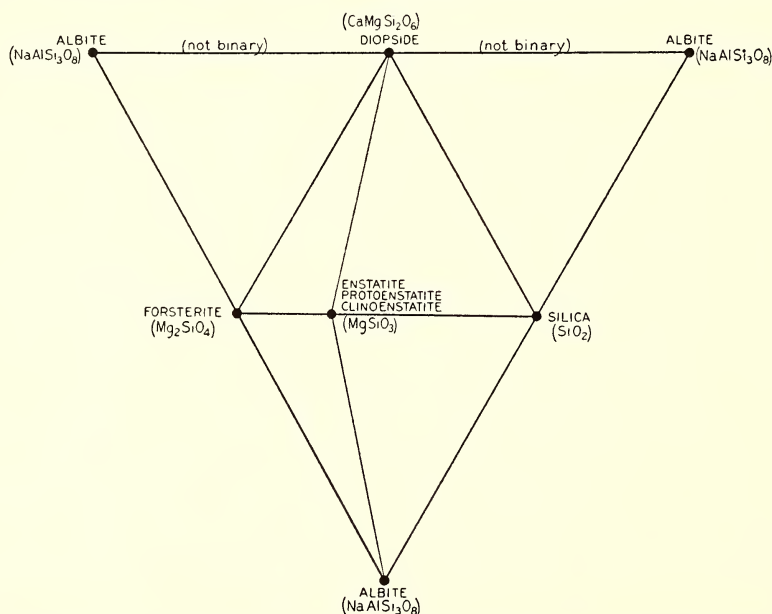


Fig. 23. Diagram showing the relations between the olivine forsterite, pyroxenes between enstatite and diopside, the soda feldspar albite, and silica.

(Year Book 56, pp. 159–160) discussed crystallization in some of the systems involved in the origin of basalts. Because of their very complexity both in crystalline modifications and in chemical composition (see Year Book 56, pp. 208–210) the rock-forming pyroxenes and pyroxenoids can yield much information on temperatures and the crystallization processes in rock formation.

During the past year we have had the opportunity to begin an important investigation of the melting relations in the system forsterite–diopside–silica–albite which involves the three mineral groups

tions in compositions between $\text{MgO} \cdot \text{SiO}_2$ and $\text{CaO} \cdot \text{MgO} \cdot 2\text{SiO}_2$.

The relation of the ternary system forsterite–diopside–silica to the system forsterite–diopside–silica–albite is shown in figure 23. The ternary system just named is shown as an equilateral triangle which is the base of a regular (equilateral) tetrahedron with the composition albite as its apex. The three faces of the tetrahedron between the base and apex have been laid flat in the plane of the base.

In addition to forsterite–diopside–silica, three other systems between this base and

the apex albite limit the system forsterite-diopside-silica-albite:

1. One of these, forsterite-albite-silica, has been studied by Greig (whose results have not yet been published), and last year Schairer (Year Book 56, pp. 217-222) gave data for the limiting binary system forsterite-albite and data for the tie line

roxene, and soda-rich plagioclase are shown. Attention is drawn to the initial rise in liquidus temperature from pure albite toward diopside, and then a falling of liquidus temperature toward the point where sodic plagioclase and diopside co-exist with liquid in the side line albite-diopside, which is not truly binary. The

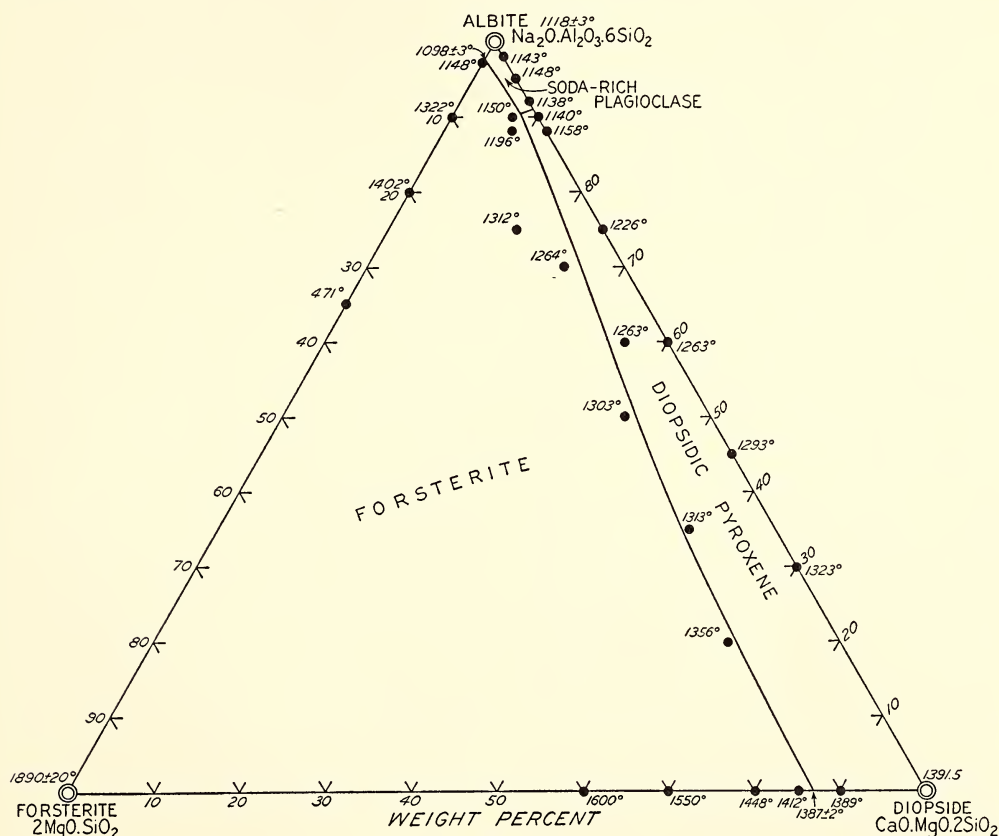


Fig. 24. Preliminary phase-equilibrium diagram of the system forsterite-albite-diopside.

albite-magnesium metasilicate as a part of his data in the system $\text{Na}_2\text{O}-\text{MgO}-\text{Al}_2\text{O}_3-\text{SiO}_2$.

2. Diopside-albite-silica is a part of the join nepheline-diopside-silica that has been studied by Schairer, but the data have not yet been published.

3. During the past year we have made a study of the system forsterite-albite-diopside, the preliminary phase-equilibrium diagram for which is given here as figure 24. The fields of forsterite, diopsidic py-

diopside crystals may not be pure diopside but may have a small Al_2O_3 content.

The triangular join MgSiO_3 -diopside-albite (not shown in fig. 24) is now under intensive investigation. So far we have prepared forty-six separate compositions, and these are being subjected to thermal, optical, and X-ray studies. Many of the melts in this important join have the olivine forsterite with two pyroxenes in equilibrium with liquids at appropriate temperatures.

PALEOBIOCHEMISTRY

P. H. Abelson

Attempts to extract organic chemicals from Precambrian rocks result in disappointingly low or negligible yields. Shales and slates of a low grade of metamorphism, such as the Keeweenawan Nonesuch shale and the Huronian Rove slate, have been examined.

After fine grinding in ball mills for 3 days, the samples were extracted with an ethyl alcohol-benzene mixture for a week in a Soxhlet apparatus. Only a small trace of soluble organic substance was found in the Nonesuch sample and nothing in the Rove slate.

It is possible that other Precambrian shales may be found that do contain extractable substances. Indeed, Swain has reported finding small amounts of organic compounds in some old rocks, including a granite. This latter occurrence is certainly adventitious.

The usual methods rarely extract more than a small fraction of the total organic matter present in sedimentary rocks. Therefore, in approaching so difficult a problem as the Precambrian sediments it seemed desirable to develop improved and essentially nondestructive methods of isolating the organic compounds present.

One possible method is hydrogenation. In general, this process does not break carbon-carbon bonds and may be carried out at temperatures below those causing excessive thermal degradation. It probably

breaks sulfur cross-linking between hydrocarbon chains and converts unsaturated aromatic compounds into more easily extracted naphthenes.

Exploratory experiments have been carried out using Kolm shale, Sweden, of Cambrian age; the Vanini shale, Nevada, of Ordovician age; and the Green River shale, Colorado, of Eocene age. The shales were ground in a ball mill for 3 days, mixed with MoS_2 , serving as a catalyst, and hydrogenated at 375°C for 5 hours at 4000 psi H_2 pressure. Spuriously high yields were obtained when a suspending medium such as tetralin was employed, and most runs were therefore made without any added organic solvent.

The solids were extracted in Soxhlet equipment for a week. Results showed that hydrogenation increased very substantially the yields from these processed shales, values of 70 per cent of the total organic matter present in the Kolm being obtained. In comparison, an aliquot of the original ground Kolm, extracted without heating, yielded 1.6 per cent, and another sample, extracted after heating at 375°C for 5 hours in a nitrogen atmosphere, gave only an 8 per cent yield. The hydrogenation procedure was thus very effective in rendering organic matter more extractable, and the approach seems sufficiently attractive to merit further investigation of its applicability to studies of organic sediments of all ages.

ORE MINERALS

During this past year systematic laboratory studies of the relations among the more common sulfide-type minerals have added greatly to our understanding of mineral associations found in nature.

Investigations of the subsolidus relations in the Cu_2S -S part of the Cu-S system and of the FeS-S part of the Fe-S system have been completed. The results have provided the necessary basic information

for systematic exploration in a number of ternary systems involving these elements. The solidus relations in the system Cu-Fe-S have been determined between 400° and 800°C . This knowledge, in combination with the data we have acquired in the Cu-S and Fe-S systems, is crucial to the work now under way on the sulfur-deficient regions of the Cu-Fe-S system.

New studies have included the more sulfur-rich parts of the Fe-Zn-S system. Thus a number of points have been determined on the curve relating temperature to composition of sphalerite formed in equilibrium with pyrite in the presence of liquid and vapor.

A detailed investigation of the phase relations in the Fe-As-S system is nearly completed. The upper stability curve of the only known ternary phase, arsenopyrite, in this system has been determined. The studies of iron, nickel, and cobalt arsenides, on which a preliminary report was given last year (Year Book 56), have been completed.

Pyrrhotite formed in equilibrium with pyrite varies significantly in composition with temperature. The experimentally determined relationship of composition to temperature has been applied to a number of ore deposits, and temperatures of formation of pyrrhotite-pyrite assemblages have been estimated. In one locality where both the sphalerite-pyrrhotite and the pyrrhotite-pyrite assemblages could be used as temperature indicators, the two methods gave substantially identical results.

Estimates of the temperature of the formation of certain sulfide veins and ore bodies by the sphalerite-pyrrhotite method have indicated the existence of strong temperature gradients during formation of the deposits, implying that such ore solutions cooled rapidly when moving away from their source, and that they may have been more concentrated than is usually thought.

Equipment for systematic studies of the solubilities of various ore-forming sulfides in aqueous solutions at pressures up to 1500 psi and at temperatures up to 200° C has been designed and constructed, and is now in routine operation. Experimental results on the solubility of sphalerite in H₂S-saturated water at various temperatures and pressures indicate solubilities six or seven orders of magnitude higher than those found for ZnS in pure water.

THE Cu-S SYSTEM

G. Kullerud

The compounds in this system are chalcocite (Cu₂S), digenite (Cu₉S₅), and covellite (CuS). Of these minerals chalcocite is most often encountered in ore deposits. Covellite is less important as an ore mineral than chalcocite but is nevertheless a common mineral in many copper ores. The third mineral, digenite, greatly resembles chalcocite in polished sections and therefore has often been incorrectly identified. During the last year X-ray diffraction studies of ore specimens from various copper sulfide deposits have shown that digenite, which possesses a very characteristic X-ray diffraction pattern, is a much more common mineral than earlier investigations employing polished sections had indicated.

Chalcocite, digenite, and covellite may be readily synthesized in the dry way in silica tubes by mixing copper with appropriate amounts of sulfur and heating the mixtures. The synthesis of covellite was described in last year's report, and the synthesis of digenite was described in a recent paper by Donnay, Donnay, and Kullerud (1958). Chalcocite forms readily from stoichiometric mixtures of copper and sulfur even at low temperatures. At 25° C, small amounts of chalcocite are formed after only a few hours. At 200° C, the reaction between copper and sulfur is rapid; after 1 day no unreacted copper or sulfur can be seen in the tubes. At 500° C and higher temperatures the reaction is extremely fast and is completed in less than 1 minute.

Figure 25 shows the relations between chalcocite, digenite, and covellite in the Cu-S system. In the diagram, vapor occurs with every phase or phase assemblage, since all experiments were performed in evacuated and sealed, rigid silica glass tubes, with a vapor phase always present. It is seen that below 507° C covellite is stable in the presence of vapor and either digenite or a sulfur-rich liquid. At 507° ±

3° C the four phases digenite+covellite+liquid+vapor are all stable, and the vapor pressure at this invariant point is about 900 mm Hg.

Numerous experiments were undertaken to determine whether covellite is

The contents of the inner tube were ground and reheated in the same way until no further change in weight was observed. (2) CuS with small amounts of sulfur was heated in evacuated tubes having a very small vapor space. This method

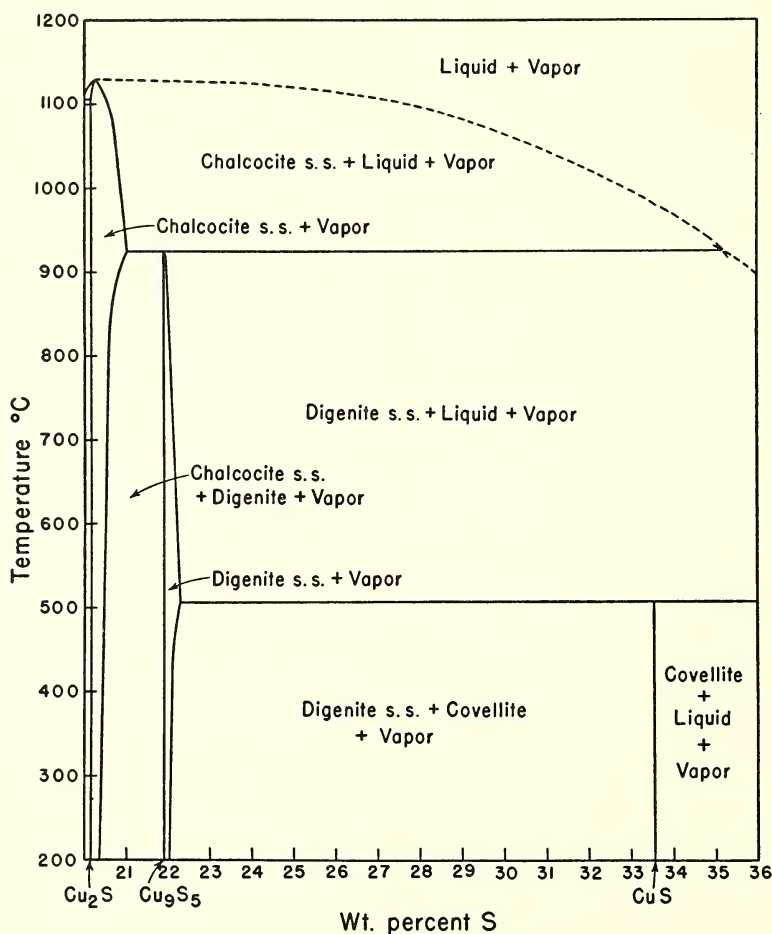


Fig. 25. Relations between chalcocite, digenite, and covellite in the Cu-S system.

capable of taking sulfur or digenite into solid solution. The maximum sulfur content of covellite was obtained by two methods. (1) A weighed amount of copper filings was heated in an open tube inside a larger closed tube containing excess sulfur; thus liquid and vapor were always present. The amount of sulfur that combined with the copper was determined by weighing the inner tube after each run.

was used only at temperatures below 400° C, where the vapor pressures are low.

Minimum sulfur content of covellite was obtained by mixing small amounts of digenite with covellite and heating the mixture in small evacuated silica tubes.

X-ray diffraction studies of phases and measurements to detect any displacement of reflections caused by possible compositional changes of covellite indicate that

covellite is a stoichiometric compound. Studies of polished sections of the products verified these findings. The copper-to-sulfur ratio of covellite lies within the range of 1 ± 0.01 .

The synthesis, as well as crystal and twin structure of digenite, was described by Donnay, Donnay, and Kullerud (1958). Further experiments on the stability of this compound have shown that digenite, which decomposes on heating to chalcocite and vapor, is stable up to about 925°C in the presence of excess sulfur (liquid+vapor). The vapor pressure at this invariant point where the four phases chalcocite+digenite+liquid+vapor all are stable has not been measured. However, it must be less than about 65 atm, which is the vapor pressure of pure sulfur at 925°C (West, 1950). This invariant point is the origin of four univariant curves, $\text{Cu}_2\text{S} + \text{Cu}_9\text{S}_5 + \text{V}$, $\text{Cu}_9\text{S}_5 + \text{L} + \text{V}$, $\text{Cu}_2\text{S} + \text{Cu}_9\text{S}_5 + \text{L}$, and $\text{Cu}_2\text{S} + \text{L} + \text{V}$, as shown schematically in figure 26. In the three univariant assemblages $\text{Cu}_2\text{S} + \text{Cu}_9\text{S}_5 + \text{V}$, $\text{Cu}_9\text{S}_5 + \text{L} + \text{V}$, and $\text{Cu}_2\text{S} + \text{L} + \text{V}$, vapor exists as a phase. The univariant curves (1), (2), and (4), therefore, can be determined by experiments employing rigid silica tubes, provided the pressures in the tubes can be measured. The fourth univariant assemblage, $\text{Cu}_2\text{S} + \text{Cu}_9\text{S}_5 + \text{L}$, does not involve a vapor phase, and, therefore, the upper stability curve (3) in figure 26 can be determined only by the use of collapsible tubes in which a vapor phase is not present. Because of extensive reaction between copper sulfide and gold at the temperatures and pressures involved, this curve could not be determined by the use of gold tubing. However, it is likely that the upper stability curve of digenite, similar to the stability curves of covellite and pyrite, is very steep. Thus, even under 30,000 psi of sulfur pressure, the breakdown temperature of digenite would probably not exceed 950°C .

The solubility of covellite in Cu_9S_5 , as well as that of chalcocite in digenite, was

studied by the methods discussed above. It was found that a digenite containing about 22.3 weight per cent sulfur was stable at 450°C . This is 0.4 weight per cent in excess of the 21.9 weight per cent sulfur contained in stoichiometric Cu_9S_5 . The solubility of chalcocite in digenite was not measurable even at 600°C . The solubility of digenite in chalcocite, on the other hand, is readily detected. Thus, at 600°C a chalcocite containing about 20.5

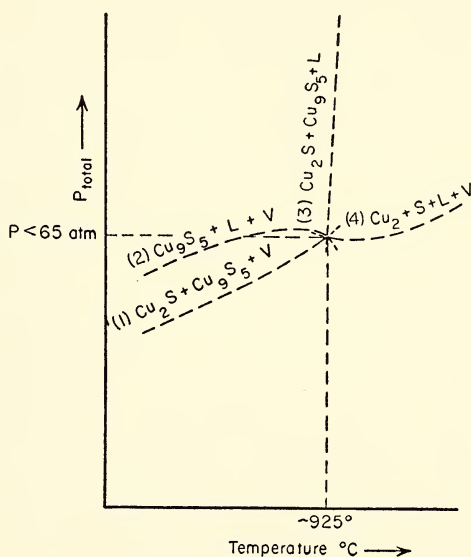


Fig. 26. Curves showing schematically invariant point where the four phases $\text{Cu}_2\text{S} + \text{Cu}_9\text{S}_5 + \text{L} + \text{V}$ are stable.

weight per cent sulfur exists in equilibrium with digenite. (Cu_2S contains 20.15 weight per cent S.) Estimates based on numerous runs indicate that the chalcocite solid solution series in equilibrium with digenite at the invariant point (about 925°C) may extend to 21.0 weight per cent sulfur.

The melting relations of chalcocite as shown in figure 25 are those determined by Jensen (1947). The dashed liquidus curves have not been determined.

Heating experiments were performed on covellite and digenite in closed evacuated tubes by the method described by Kracek (1946). Covellite showed no phase

changes between 25° and 490° C, where breakdown occurred. Runs on digenite indicated an inversion at 65° C, but no other effects of heat between 65° and 650° C were noted.

THE Fe-S SYSTEM

R. G. Arnold

During the past year experimental work on the pyrrhotite-pyrite relations has been completed, and experiments to determine the location of the curve representing the composition of liquids that can coexist with pyrrhotite and vapor have been initiated. The applicability of the experimental $d(102)$ -value/composition relation to natural pyrrhotites has been investigated over a limited composition range. Temperatures of formation of a number of coexisting pyrrhotite-pyrite assemblages have been estimated.

The curve representing the composition of pyrrhotite formed in equilibrium with pyrite at the pressure of the coexisting vapor has been extended from 650° C to the incongruent melting temperature of pyrite at 743° C. The extended portion of this curve was found to be a smooth continuation of the curve previously determined up to 650° C presented in last year's report (Year Book 56, p. 192, fig. 24). Thus the earlier evidence for a pyrrhotite inversion at $670 \pm 5^\circ$ C has disappeared.

To determine this curve above 650° C the procedure was as follows. Charges consisting of approximately 2.5 g of pelleted sulfide (pyrrhotite and pyrite) of known bulk composition were fitted snugly into the bottom of sealed silica glass tubes. Silica glass rods and silica glass powder were used to reduce the vapor space to a minimum. All charges were heated to 755° C for several hours to decompose any pyrite present, then held at specified temperatures for 84 hours or longer. Microscopic examination of the products permitted distinction of stable pyrite from exsolved pyrite and from pyrite formed by reaction

between pyrrhotite and vapor during quenching. Rapidly exsolved pyrite was dispersed throughout the pyrrhotite parent in grains or plates about 6 μ or less in thickness. Pyrite rimming the pyrrhotite grains was formed by the reaction of pyrrhotite with vapor on quenching and occurred as grains about the same size as exsolved pyrite. Stable pyrite, however, was present in much larger grains ($\geq 20 \mu$) situated at or near the borders of pyrrhotite grains. The curve representing the composition of pyrrhotite on the pyrrhotite-pyrite solvus was bracketed by points representing the bulk composition of charges in which stable pyrite was present or absent.

The initial compositions of the charges were corrected for loss of sulfur to the vapor by means of the familiar relation $PV = nRT$. The molecular weight of sulfur was calculated from the data summarized by West (1950). The vapor pressures were estimated from the extrapolated data of Allen and Lombard, De Rudder, D'Or, Raeder, Juza and Biltz, and Rosenqvist.

The resulting solvus curve is shown in figure 27. The composition attained by extrapolating the curve to 743° C is 44.9 atomic per cent Fe. The uncertainty in these compositions is about ± 0.1 atomic per cent Fe. The position of the curve below 650° C was checked at 600° and 400° C by the sintering and microscopic method described above.

The lamellar phase described and shown in last year's report (Year Book 56, fig. 25) was repeatedly identified in pyrrhotites rapidly quenched from above 666° C and initially more sulfur-rich than 45.65 atomic per cent Fe. X-ray powder photographs of pyrrhotite containing about 20 volume per cent lamellae gave a series of reflections that could not be attributed to either pyrite, marcasite, the parent hexagonal pyrrhotite, monoclinic pyrrhotite, smythite (Fe_3S_4), or kansite (Fe_9S_8). The five most prominent reflections attributed to the lamellar phase gave the following d values: 5.648, 5.346

4.999, 2.562, 1.980 Å. The symmetry of the crystal structure and the field of stability of this lamellar phase have not been determined.

The determinative curve relating the $d(102)$ values of pyrrhotite solid solutions

their $d(102)$ values) of pyrrhotites from charges whose compositions were more sulfur-rich than 46.0 atomic per cent Fe. The subsequent detection of exsolved pyrite in these pyrrhotites, however, indicates that the true limit of solid solution must

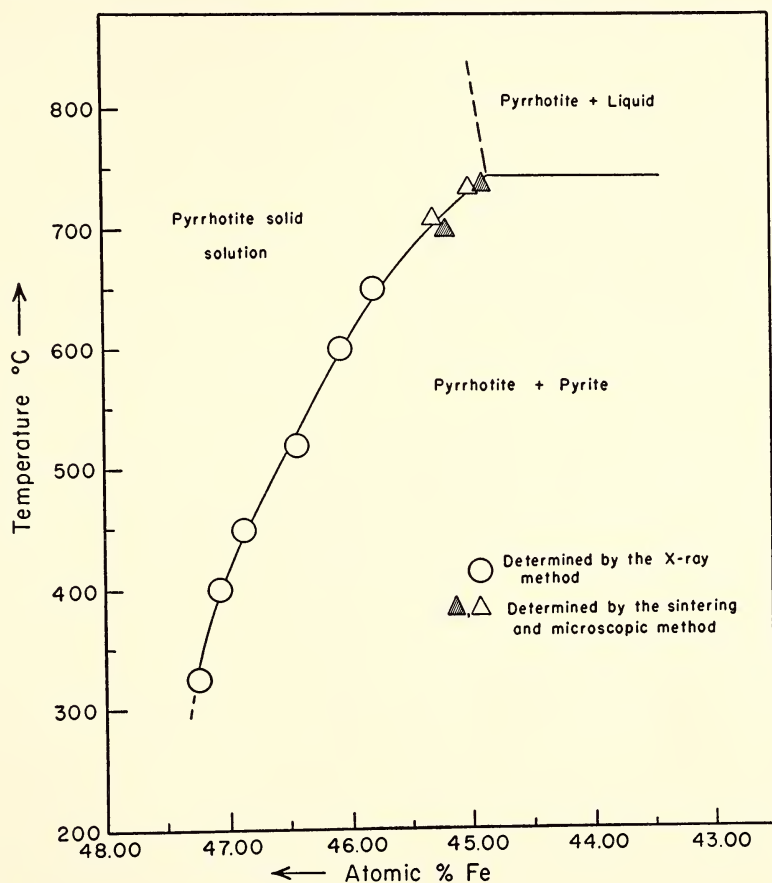


Fig. 27. A portion of the FeS-S equilibrium diagram as it would look at a pressure of about 10 bars. Circles represent pyrrhotite compositions determined by the X-ray method. Open and hatched triangles represent the bulk compositions of runs containing the condensed phases pyrrhotite, and pyrrhotite and pyrite, respectively. Data points have been projected on this isobaric section.

synthesized at 800° C to their iron contents was presented in Year Book 55 (p. 177). The composition (46.0 atomic per cent Fe) designated as the most sulfur-rich solid solution attainable at this temperature is believed to be incorrect. This limit of solid solution was based on the similarity of the iron contents (judged by the constancy of

lie somewhat to the sulfur side of the reported limit of solid solution. Pyrrhotite solid solutions less sulfur-rich than 46.0 atomic per cent Fe contained no detectable pyrite; hence, the determinative curve, based on the measurement of these pyrrhotites, is believed to be correct.

The $d(102)$ -value/composition relation

has been studied further by measuring the $d(102)$ values of pyrrhotite solid solutions synthesized at 600° and 400° C. The agreement of these data with those obtained from preparations synthesized at 800° C indicates that the $d(102)$ -value/composition relation is independent of the temperature at which the preparations are synthesized.

The possibility of applying the $d(102)$ -value/composition relation to natural pyrrhotites (containing up to 0.4 per cent combined cobalt and nickel in solid solution) has been investigated over the composition range 46.5 to 47.7 atomic per cent Fe. The iron contents of six pyrrhotites determined by chemical analysis were within ± 0.1 atomic per cent Fe of the iron content as indicated by the X-ray method.

The temperatures of formation of a number of pyrrhotite-pyrite pairs (containing ≤ 0.15 weight per cent combined nickel and cobalt) from mineral deposits were estimated at arbitrarily assumed total pressures of 1000 and 2000 bars, approximately the pressures exerted by 2.5 and 5 miles of rock cover, respectively. The iron contents of all but one pyrrhotite, which was determined by chemical analysis, were determined by the X-ray method. The temperatures of formation were estimated using the solvus curve determined at 1000 and 2000 bars total pressure which was presented in last year's report (fig. 26, p. 194). Table 8 gives the iron contents of each pyrrhotite, their estimated temperatures of formation, and a brief statement of the type of deposit involved. The iron content of sphalerite coexisting with pyrrhotite and pyrite in the Heath Steele Mine indicated a temperature of formation of about 550° C at 2000 bars using Kullerud's (1953) data for the FeS-ZnS system. Pyrrhotite formed in equilibrium with pyrite in the same specimen also gives 550° C (see table 8).

Figure 28 shows the distribution of the iron contents of 61 natural pyrrhotites

plotted against the number of measurements in each $\frac{1}{4}$ atomic per cent Fe range. This compilation includes the results of chemical analysis obtained from the literature and from the present study, as well as the X-ray analysis of material containing ≤ 0.4 per cent combined cobalt and nickel. Chemical analysis was recalculated to 100 per cent where required. All but one of these pyrrhotites, a troilite, are of terrestrial origin and come from base-metal deposits, pegmatites, schists, and basic igneous rocks. The iron contents of these pyrrhotites fall into two distinct groups, designated A and B, the majority of measurements being in group B. The pyrrhotites of group B were generally associated with pyrite or chalcopyrite or both, whereas only one pyrrhotite of group A showed a trace of any other sulfide.

The curve representing the composition of sulfur-rich liquids that can coexist in equilibrium with pyrrhotite solid solution and vapor above 743° C is being determined. Charges consist of sulfur and a single grain of iron (converted to pyrrhotite above 743° C) sealed in evacuated bent silica tubes. A single grain was used in preference to a powder to decrease the difficulty of separating the solid phase from the sulfur-rich phase in the completed run. Each tube was rotated at 1 rpm in a vertical plane in the furnace for up to 6 days to facilitate intimate contact between the single pyrrhotite lump and all portions of the sulfur-rich liquid. Before retracting a completed run for slow air quenching, each tube was tipped so that the single pyrrhotite lump remained in the short arm of the bent tube while the liquid drained into the long arm. After quenching, the contents of the long arm consisted largely of a sulfur-rich crystalline phase (containing small sulfide crystals precipitated during cooling) representing original liquid, which was capped by a virtually pure sulfur phase representing condensed vapor. The two phases were separated, and the sulfide-containing

TABLE 8. Estimated Temperature of Formation of a Number of Ore Deposits Containing Pyrrhotite and Pyrite.

No.	Deposit and Location	Pyrrhotite Composition *	Temperature of Formation, ° C		Geological Setting
			P = 1000 bars	P = 2000 bars	
1	Lucky Strike Mine, Colo.	46.40	560	610	Replacement of crystalline limestones
2	Bicroft Uranium Mine, Ont.	46.51 †	540	585	Vein in uraniferous pegmatite cutting biotites paragneiss
3	Heath Steele Mine, N. B.	46.62	515	550	Replacement of acid and intermediate volcanic tuffs and flows
4	Aldermac Mine, Que.	46.66	510	540	Replacement of Keewatin volcanics
5	East Sullivan Mine, Que.	46.64	510	540	Massive sulfide replacement of Precambrian volcanics
6	Clearwater Brook, N. B.	46.72	490	520	Replacement of siliceous metasedimentary rocks
7	Highland Surprise Mine, Ida.	47.00 – 46.60	420 – 515	425 – 555	Replacement of fractured zone in siliceous argillite and quartzite
8	Brunswick Mine, N. B.	46.78	455	495	Massive sulfide replacement of siliceous metasedimentary rocks
9	Burra Burra Mine, Tenn.	46.86	440	470	Massive sulfide replacement of crystalline limestones

* The uncertainty in these compositions determined by the X-ray method is not known. However; a comparison of chemical analysis and X-ray analysis in 6 instances described in the text indicates agreement to ± 0.1 atomic per cent Fe. Until more information is obtained the uncertainty in these compositions is taken to be ± 0.1 atomic per cent Fe.

† Determined by chemical analysis. X-ray analysis gave 46.58 atomic per cent Fe. Analyst, M. K. Carron. Job 3723.

Source of Material

No. 1 A. H. Koschmann, U. S. G. S.

No. 2 P. K. Cunningham-Dunlop, Princeton University.

No. 4 #3432 McGill University collection.

No. 5 J. R. Assad, McGill University.

No. 6 W. Petruk, McGill University.

No. 7 R. G. Coleman and V. C. Fryklund, U. S. G. S.

No. 9 C. S. Ross, U. S. G. S.

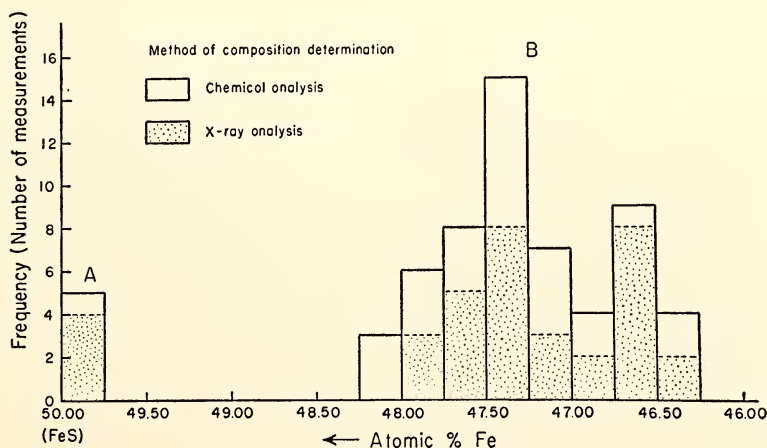


Fig. 28. Diagram showing the distribution of iron content of 61 pyrrhotites. The height of each column indicates the number of each kind of measurement.

phase was analyzed for iron. The solubility of iron in sulfur liquid even at 895° C was found to be ~0.1 weight per cent Fe.

THE SOLIDUS IN THE SYSTEM Cu-Fe-S
BETWEEN 400° AND 800° C

E. H. Roseboom, Jr., and G. Kullerud

Of great significance to the mining industry is the system Cu-Fe-S, which contains most of the sulfides found in a large number of ore deposits. Although numerous workers have investigated various parts of the system, the phase relations in the sulfur-rich portion have received little attention. Recently Kullerud has studied the sulfur-rich part of the system Cu-S, and Arnold, as well as Kullerud and Yoder, has investigated the Fe-S join. The study most closely related to the present work is that of Merwin and Lombard (1937).

In the system Cu-Fe-S, a sulfur-rich liquid phase persists down to a temperature probably only slightly lower than the melting point of sulfur (114.5° C for pure S). The present work is an investigation of the solid phases in equilibrium with this liquid plus a vapor phase between 400° and 800° C.

Mixtures of synthetic phases or of copper and iron metals were heated in sealed evacuated silica glass tubes containing enough sulfur to provide a liquid as well as a vapor phase. Above 550° C equilibrium was attained in a few hours. Above 565° C, mixtures of copper, iron, and sulfur were used as starting materials; below 565° C, mixtures of covellite, pyrite, and sulfur. The temperatures of invariant points below 565° (fig. 29; points 1, 2, and 3) were located by taking two samples, one that had previously been annealed to produce the stable assemblage above the invariant point under investigation and another that had been similarly annealed below that invariant point, and heating them in separate tubes side by side. Thus, equilibrium was approached from both higher and lower temperatures. Near the

invariant point (fig. 29, point 1) at 434° C, runs of 1 month produced only about 5 per cent reaction, but the direction was clearly established from the pairs of runs. The solid phases were identified by means of polished sections and by X-ray diffractometer methods.

The results of these experiments cannot be depicted on a simple isothermal or isobaric section because the vapor pressure increases as the temperature is raised. Although the experimental work as described is simple, the *P-T* conditions in the part of the system studied are rather complex. In order to relate the portion of the system studied to other experimental work we shall first consider a *P-T* projection of a *P-T-X* diagram covering a wider range of conditions than those actually studied (fig. 29).

The curve extending steeply downward from point 4 is the dissociation curve for covellite going to digenite plus a sulfur-rich vapor in the binary system Cu-S. Similarly the curve extending steeply downward from point 7 is the dissociation curve for pyrite going to pyrrhotite plus a sulfur-rich vapor in the binary system Fe-S. In each case, as the dissociation curve is crossed at constant pressure going toward a higher temperature, a solid phase dissociates into a solid phase lower in sulfur plus a sulfur-rich vapor. As the reactions occurring on these curves each involve three phases in two-component systems, the dissociation curves represent univariant conditions.

The curves extending steeply downward from points 1, 2, 3, 5, and 6 are univariant curves for three solids plus a vapor in the ternary system Cu-Fe-S. They are analogous to the dissociation curves in the binary systems except that one additional solid phase is always present. They are of two general types: (1) $A+B \rightleftharpoons C+V$, (2) $C \rightleftharpoons A+B+V$. *A*, *B*, and *C* are sulfides, and *V* is a sulfur-rich vapor. The curves from points 1, 2, 5, and 6 are of the first type, and the curve from point 3 is the second type.

If any one of these univariant curves is followed to higher temperatures and pressures, it is seen to terminate at a point where the vapor phase begins to condense, and thus a fifth phase, a sulfur-rich liquid, appears. The temperature and pressure at which this occurs represent an invariant point on a P - T projection, because there are now five phases in a three-component system and there are no degrees of freedom

vertical curves represent the same reactions as the curves extending steeply downward from the invariant points, except that the sulfur-rich vapor has been replaced by a sulfur-rich liquid in the system.

We have already discussed two of the five univariant curves. The remaining three do not involve the formation or decomposition of a solid phase, but are

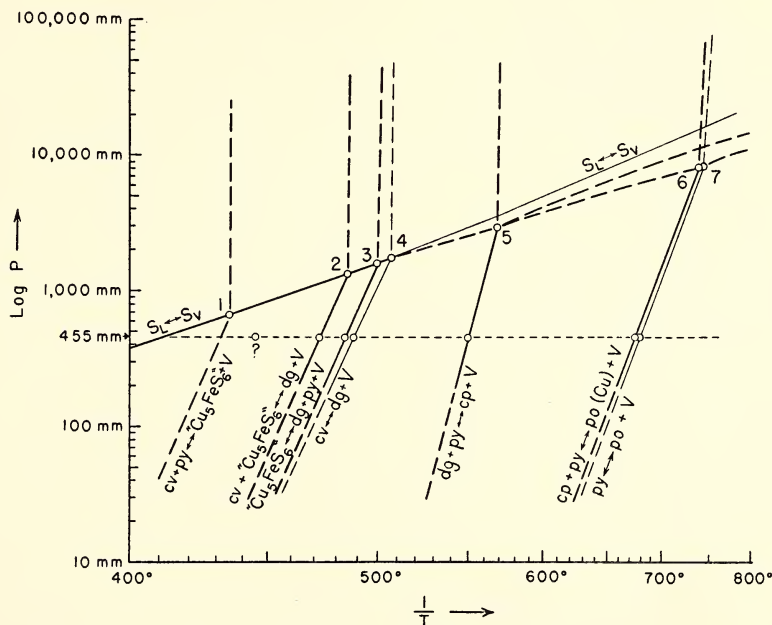


Fig. 29. Pressure-temperature projection of a part of the system Cu-Fe-S. The points on the horizontal dashed lines are from Merwin and Lombard's 455 mm isobaric section. cv, covellite; py, pyrite; dg, digenite; po, pyrrhotite; cp, chalcopyrite; S_L , sulfur liquid; S_V , sulfur vapor.

remaining. The ternary invariant points are numbers 1, 2, 3, 5, and 6 in figure 29.

Each of these invariant points represents the point of origin of five univariant curves,⁴ four of which involve a vapor, and a fifth which does not. From each invariant point the last is shown as a nearly vertical dashed line extending toward regions of higher pressures. These

⁴ Three of the univariant curves emanating from each of points 1, 2, and 3 are probably nearly coincident with the curve labeled S_L - S_V and are not shown as separate curves. These are the curves representing the P - T conditions of the present work.

simply curves along which both a sulfur-rich liquid and a vapor are in equilibrium with two solid phases. These curves connect the various invariant points, and the pairs of solids that are stable together with liquid and vapor along them are indicated in figures 30 and 31. On the high-pressure, low-temperature side of these curves, the two solids are stable with a sulfur-rich liquid; on the low-pressure, high-temperature side, the same two solids are stable with a sulfur-rich vapor. Some of these solid-solid-liquid-vapor curves approximately coincide with one another and with

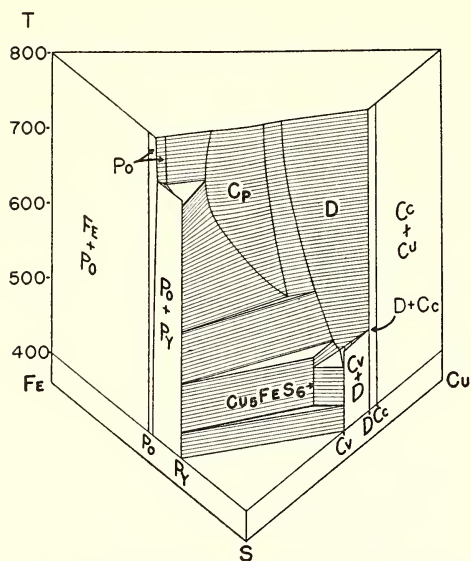


Fig. 30. The solidus (in the presence of a vapor) in the system Cu-Fe-S between 400° and 800° C. Same abbreviations as preceding figure except: D, digenite; Cc, chalcocite.

the liquid-vapor curve for sulfur at low temperatures. For example, running from point 1 toward points 2 and 3 there are actually two curves of this type. One represents the vapor pressure over a sulfur-

rich liquid, covellite, and "Cu₅FeS₆." The other represents the vapor pressure over liquid, "Cu₅FeS₆," and pyrite. Over this *P-T* range it is unlikely that sufficient copper or iron goes into the liquid or vapor phase to cause the vapor composition, and consequently the vapor pressure, to be appreciably different from those of sulfur. Consequently, these two curves coincide with one another and also with the liquid-vapor curve for pure sulfur.

The line labeled *S_L-S_V* is the liquid-vapor curve or vaporization curve for pure sulfur. At low temperatures this curve coincides with the solid-solid-liquid-vapor curves because the liquid and vapor phases in equilibrium with the solid phases are very close in composition to pure sulfur. At higher temperatures, sufficient amounts of iron or copper or both may be dissolved in the liquid and the vapor phases to cause the solid-solid-liquid-vapor curves to shift appreciably from the sulfur vaporization curve. For this reason the solid-solid-liquid-vapor curves are shown as having lower vapor pressures than the sulfur vaporization at temperatures above 500° C.

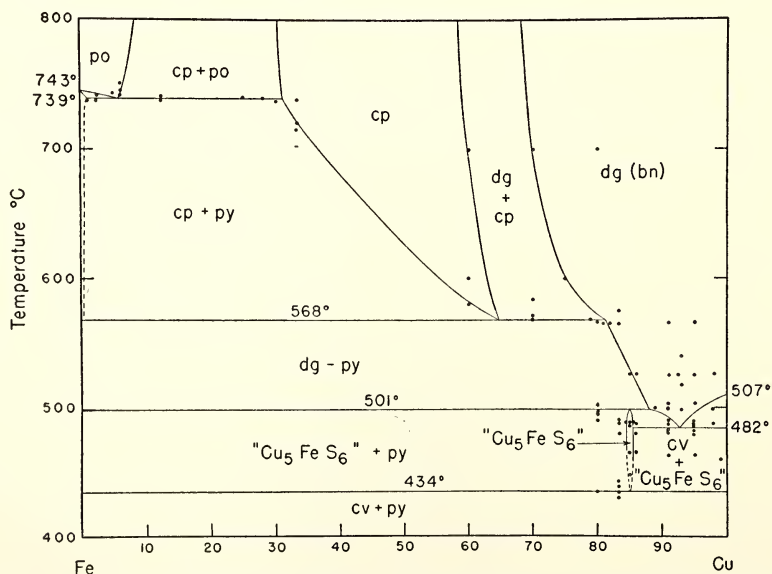


Fig. 31. The solidus in the system Cu-Fe-S as seen from the S corner, i.e. projected onto the Fe-Cu side of figure 30.

Experiments conducted by Rosenqvist (personal communication, 1957) and later by Arnold (1958) on the solubility of iron in liquid sulfur indicate small solubilities (<0.1 per cent at 800°C), suggesting that the pressure difference between the total vapor pressure over pure sulfur and over sulfur saturated with iron might be small. Extrapolation by Kullerud and Yoder (1957) of the known data on dissociation of pyrite to pyrrhotite and vapor to the invariant point at 743°C , where pyrrhotite, pyrite, liquid, and vapor all are stable, gives a total pressure of about 10 atm. On the other hand, the vapor pressure over pure sulfur at 743°C is given by West (1950) as about 21 atm.

Merwin and Lombard constructed a "constant pressure" section at 455 mm and made various measurements of dissociation pressures. They encountered on their section the various dissociation curves at the temperatures indicated in figure 29, on the line at 455 mm.

The present work is not a constant-pressure section but follows along the solid-solid-liquid-vapor curves and passes through all the invariant points shown. With respect to the solid phases only, such a section is bound to resemble Merwin and Lombard's because their section crosses the univariant curves that originate at these invariant points.

Figure 30 shows the solid phases that exist in equilibrium with the sulfur-rich liquid and vapor in the system. The liquid and vapor are not shown, but are understood as lying near the sulfur corner and being stable with all the phases shown. Thus, below 434°C , pyrite and covellite are stable with the liquid and vapor. At 434°C (invariant point 1 in fig. 29), pyrite, covellite, and Cu_5FeS_6 are stable together. Immediately above 434°C , pyrite and Cu_5FeS_6 are stable, as are Cu_5FeS_6 and covellite. The latter pair are stable together up to 482°C (invariant point 2, fig. 29). At this temperature, a digenite with about 7 per cent iron substituting for copper is also stable. Immediately

above this temperature, a digenite lower in iron is stable with covellite and a digenite higher in iron is stable with Cu_5FeS_6 . The higher-iron digenite becomes richer in iron with rising temperature until at 501°C the Cu_5FeS_6 breaks down. Above 501°C , the iron-rich digenite is stable with pyrite. It becomes richer in iron with increasing temperature. At about 550°C , there is a complete solid solution between bornite and digenite.

The low-iron digenite that became stable with covellite at 482°C becomes lower in iron with rising temperature until it becomes pure digenite at 507°C (invariant point 4, fig. 29), which is an invariant point in the binary system Cu-S. At 568°C the digenite-bornite solid solution is stable with pyrite and with a copper-rich chalcopyrite (invariant point 5, fig. 29). Above 568°C the digenite-bornite solid solution is stable with a copper-rich chalcopyrite, and a chalcopyrite which becomes progressively richer in iron with rising temperature is stable with pyrite. At 739°C the latter pair are stable with a pyrrhotite containing about 5 per cent copper substituting for iron (invariant point 6, fig. 29). Immediately above 739°C chalcopyrite (of about cubanite composition) is stable with the copper-bearing pyrrhotite and a copper-bearing pyrrhotite is stable with pyrite. This last pyrrhotite becomes lower in copper with rising temperature, until at 743°C (invariant point 7, fig. 29) pyrite breaks down and pure iron pyrrhotite becomes stable. Above this temperature three solid solutions are stable: a copper-bearing pyrrhotite, a chalcopyrite solid solution containing Cu and Fe in ratios variable from about Cu_3Fe_7 to Cu_6Fe_4 , and a digenite-bornite solid solution. The copper-rich pyrrhotite is stable with the iron-rich chalcopyrite, and the copper-rich chalcopyrite is stable with the iron-rich digenite. It should be remembered that all the above solid phases are stable with both a sulfur-rich liquid and a sulfur-rich vapor.

Figure 31 is a projection of the surface

shown in figure 30 as seen from the sulfur corner. On this diagram are plotted the Cu:Fe ratios of the samples and the temperatures at which they were heated. The various regions indicate what sulfide phases were encountered in the runs, all of which contained a sulfur-rich liquid and a vapor. The sulfur contents of the various phases could not be determined, as they could not be separated from the liquid and condensed vapor; moreover, on the copper-rich side some covellite or " Cu_5FeS_6 " or both frequently form on quenching from reaction between the digenite-bornite phase and liquid or vapor. It seems likely, however, that the sulfur contents are about the same as those determined by Merwin and Lombard.

This study indicates the maximum temperatures at which some of these mineral assemblages can exist in the presence of a sulfur-rich vapor. Thus, covellite and pyrite cannot exist together above 434°C , although covellite alone can exist up to 507°C and pyrite alone up to 743°C . A list of such assemblages and corresponding maximum temperatures in the presence of sulfur-rich vapor follows: covellite + pyrite, 434°C ; covellite + " Cu_5FeS_6 ," 482°C ; pyrite + " Cu_5FeS_6 ," 501°C ; bornite + pyrite, 568°C ; chalcopyrite + pyrite, 739°C .

With no vapor present, and under confining pressures greater than those existing at the various invariant points, these assemblages would break down at higher temperatures than those listed to one or two solids plus a sulfur-rich liquid. Kullerud's work on dissociation of covellite, as well as the work by Kullerud and Yoder on dissociation of pyrite, at high pressures where vapor was absent showed that the stability fields of these two minerals were increased by about 10° to 15°C per 1000 bars of pressure, and changes of a similar order of magnitude might be expected for other dissociations of sulfides or assemblages of sulfides.

The study also showed that when liquid and vapor are present digenite and bornite

form a complete solid solution above about 550°C . The first digenite to appear with rising temperature was one nearly midway in the series digenite-bornite; it was observed at 482°C . This same phase appeared in Merwin and Lombard's work at 472°C at 455 mm but was identified as chalcocite from its appearance under the microscope. Thus, the crest of any solvus between digenite and bornite is probably below this temperature. The existence of such a solvus is often indicated in ore deposits where digenite and bornite occur together as separate phases. The assemblage chalcocite-pyrite has been described from many localities. Such an assemblage is incompatible with the tie line between digenite and bornite found under the conditions of the present study but may be stable in parts of the system that have not been investigated.

The phase Cu_5FeS_6 , first described by Merwin and Lombard, had a Cu:Fe ratio of 17:3 under the P - T conditions of the present study. This phase has never been definitely identified in any natural deposits, although its strong pleochroism and intense anisotropism make it very easy to observe in polished sections. The assemblage covellite + pyrite (which would occur at lower temperatures and higher vapor pressures) and the assemblage bornite (digenite) + pyrite (which would occur at higher temperatures and lower vapor pressures) are both known in ore deposits. Merwin and Lombard heated " Cu_5FeS_6 " for 5 days at 300°C and 200°C , 8 days at 100°C , and 15 days at 87°C , and it showed no sign of breaking down. The failure of this phase to appear in nature remains to be explained.

The phase labeled chalcopyrite in figure 31 extends over a wide Cu:Fe range (at 800°C from about $\text{Cu}_{58}\text{Fe}_{42}$ to $\text{Cu}_{30}\text{Fe}_{70}$), beyond the ratio of 1:2 of the mineral cubanite. The X-ray pattern obtained for this composition range is that of chalcopyrite. Thus, since there is a continuous solid solution between chalcopyrite and cubanite composition, under these condi-

tions cubanite must invert to the chalcopyrite-type structure at some temperature below that at which the solid solution series is complete. From figure 31 it is noted that under the conditions of these experiments cubanite must possess the chalcopyrite structure at least above 720° C.

THE Fe-Zn-S SYSTEM

P. B. Barton, Jr.,⁵ and G. Kullerud

The composition of mix-crystals in the FeS-ZnS binary system (Kullerud, 1953) has been extensively used in geological thermometry. For this purpose the im-

sphalerite (+vapor) field (extending to point *a* in fig. 32*a*), which contains no measurable excess of sulfur. Pyrite is essentially stoichiometric (Kullerud and Yoder, 1958), and the liquid field is almost pure sulfur (less than 0.1 per cent Fe+Zn can dissolve in sulfur at this temperature). The tie lines of the three-phase fields Po+Sp+V, Py+Sp+V, and Sp+L+V represent lines of equal vapor pressure, and the vapor pressure across each of these fields increases toward the more sulfur-rich compositions.

Geologically, we are limited to those

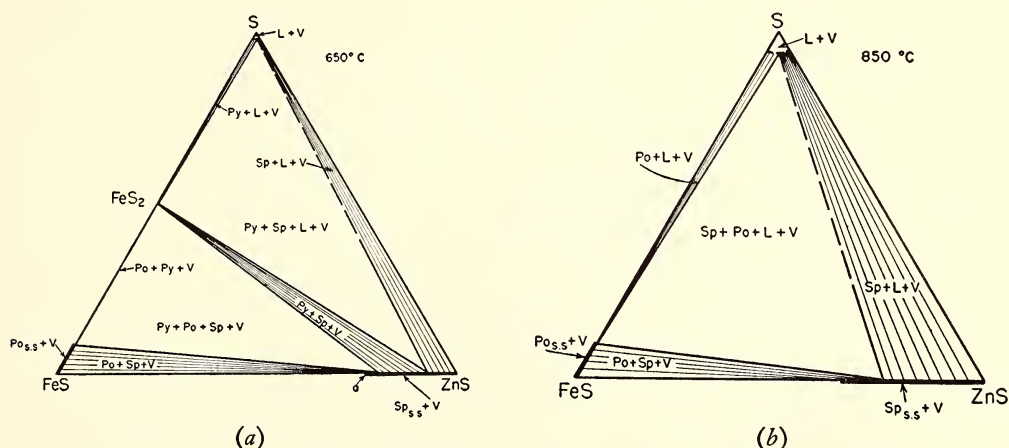


Fig. 32. Phase relations in the FeS-ZnS-S part of the Fe-Zn-S system (a) at 650° C, (b) at 850° C.

portant part of the binary join is the solvus curve that defines the iron content of sphalerite saturated with FeS as a function of temperature. The natural iron sulfides (pyrite and pyrrhotite) commonly found with sphalerite are richer in sulfur than stoichiometric FeS, and, therefore, the more sulfur-rich part of the Fe-Zn-S ternary system must be considered.

Figure 32*a* shows the FeS-ZnS-S part of the ternary Fe-Zn-S system at 650° C. Two of the solid solution series, shown by heavy lines, on the bounding joins are for all practical purposes binary: the pyrrhotite (+vapor) field, which contains essentially no zinc (Kullerud, 1953), and the

fields that do not contain liquid sulfur, because sulfur rarely, if ever, accompanies sphalerite as a hydrothermal mineral. The experiments so far have been designed to investigate the two univariant fields containing the assemblages Py+Po+Sp+V and Py+Sp+L+V. At 650° C a difference between the composition of sphalerite in equilibrium with pyrrhotite and that in equilibrium with stoichiometric FeS was found to exist. Figure 33 shows the variation in the iron content of sphalerite as a function of the phase assemblage and temperature. Because the composition of the (Fe,Zn)S mix-crystals falls essentially on the FeS-ZnS binary join, the experimental points from the ternary part of the system have been projected on the plane

⁵ U. S. Geological Survey, publication approved by Director.

of the FeS–ZnS binary. The new experimental points are shown by a bar indicating the uncertainty in the composition of the sphalerite.⁶ In figure 33 the curve marked FeS+Sp+V is Kullerud's original FeS–ZnS solvus curve. The curve marked Sp+Py+L+V gives the composition of sphalerite in equilibrium with pyrite as well as liquid and vapor. Pure pyrite melts incongruently at 743° C (Kullerud

between 500° and 600° C. Kullerud's curve is thus applicable to any pyrrhotite–sphalerite or pyrrhotite–pyrite–sphalerite assemblage below this temperature.

The central area in figure 33 is that of Py+Sp+V. It is seen that a sphalerite which contains 10 mole per cent FeS and which formed in equilibrium with pyrite could have been deposited at any temperature between 340° and 680° C. Points

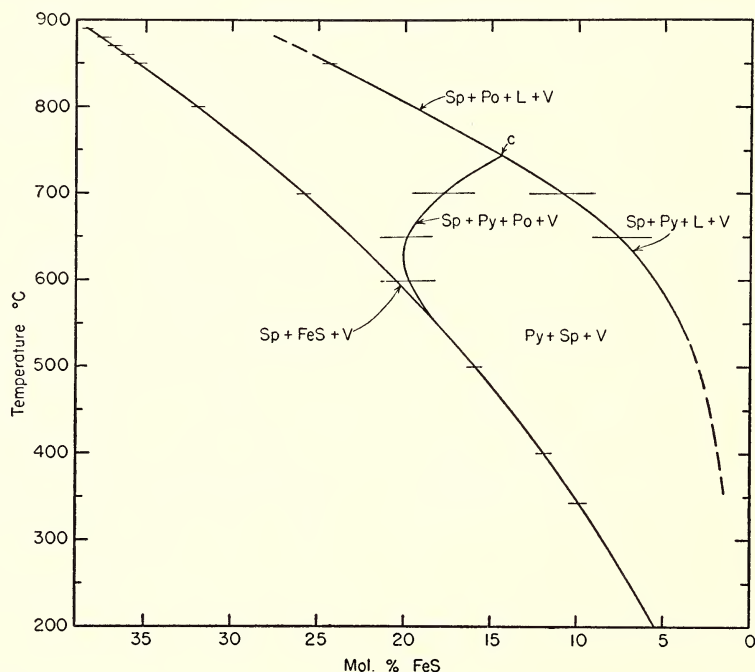


Fig. 33. Variation in iron content of sphalerite as a function of phase assemblage and temperature.

and Yoder, 1957). Above this temperature sphalerite exists in equilibrium with pyrrhotite, liquid, and vapor as shown in figure 32*b*. The Sp+Py+Po+V curve represents the composition of sphalerite in equilibrium with pyrite, pyrrhotite, and vapor. It is seen that the curves Sp+FeS+V and Sp+Py+Po+V diverge above 600° C and for purposes of practical measurement coincide at some temperature

⁶ A new curve relating the iron content of synthetic sphalerites to their cell dimensions was established by use of the Norelco X-ray diffractometer with Fe radiation and Mn filter, using CaF₂ as internal standard.

within this area are determined by the sulfur pressure over the assemblage at the time of its formation. The iron content of sphalerites deposited in equilibrium with pyrite where no pyrrhotite is present, therefore, gives a minimum temperature of formation of the sphalerite–pyrite assemblage, when the binary FeS–ZnS solvus curve is used as a temperature indicator. Similarly, a “maximum” temperature is obtained if the curve is used that relates composition of sphalerite existing in equilibrium with pyrite and liquid (+ vapor). In order to specify at which temperatures, between these limits, natural

sphalerite-pyrite assemblages were deposited, the vapor pressure of sulfur during formation as well as the relations between iron content of sphalerite and sulfur pressure must be known. Whereas the partial vapor pressure during deposition of sphalerite-pyrite assemblages can often be estimated from coexisting sulfides, the solvus curves relating composition of sphalerite to sulfur pressure are being studied experimentally.

common. In addition, the system contains the phases FeAs and Fe₂As, of which no natural occurrences are known.

Knowledge of the phase relations among minerals containing arsenic is applicable to a number of important ore deposits. Hence a systematic study of these arsenides was initiated early this year and is now nearly completed. The work was done in evacuated, sealed, silica glass tubes, where a vapor was always present. In all runs

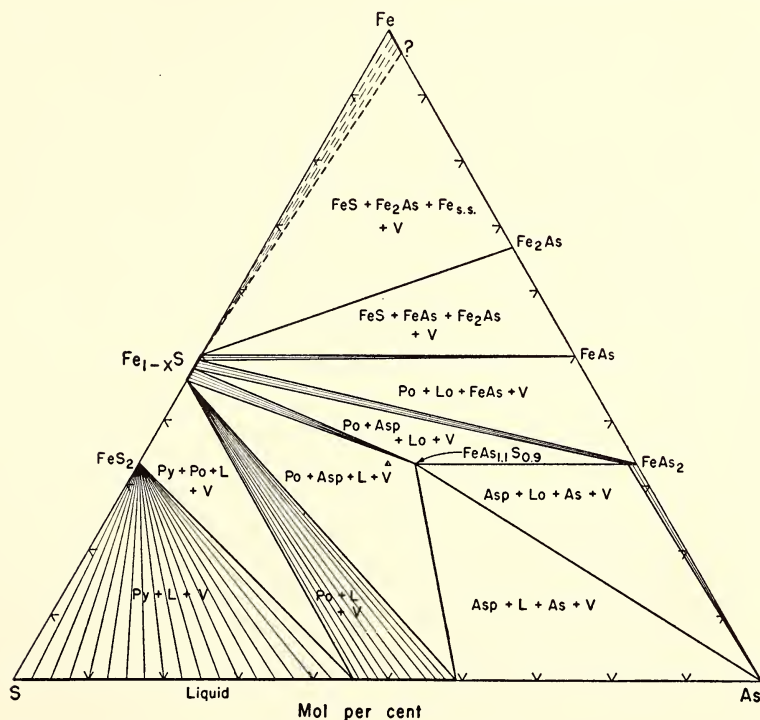


Fig. 34. Phase relations in the Fe-As-S system at 600°C. Vapor pressures are those of the system. FeAsS composition is marked by the small triangle at center.

THE Fe-As-S SYSTEM

L. A. Clark⁷

Minerals of this system occur in most known sulfide deposits. The common minerals of the group are pyrite (FeS₂), pyrrhotite (Fe_{1-x}S), and arsenopyrite (FeAsS). Loellingite (FeAs₂), realgar (AsS), and orpiment (As₂S₃) are less

the vapor volume was reduced to such an extent that the change in bulk composition of the condensed phases due to its presence was not more than 0.25 weight per cent; usually it was much less. Vapor pressure varies with changes in the coexisting phases, and is constant in each four-phase assemblage at constant temperature. It increases from a few millimeters in the Fe-rich assemblages to a few atmospheres along the S-As side of the diagram (fig.

⁷ Fellow of the National Research Council of Canada.

34) as estimated from known vapor pressures of Fe, S, As, and Fe-S assemblages.

The S-As minerals orpiment and realgar melt at slightly above 300° C, and so at 600° C there is a continuous liquid field extending along the S-As binary from 100 to 22.8 ± 0.2 weight per cent sulfur. Since less than 0.1 per cent iron is soluble in the liquid at this temperature, the ternary

component. The solubility of sulfur in loellingite is greater than 3 weight per cent at 700° C, but appears to be less than 1 per cent at 600° C. At the latter temperature several months are required to attain equilibrium. Work has not been completed on the composition of synthetic arsenopyrite. There are three indications that it is As-rich, with an approximate

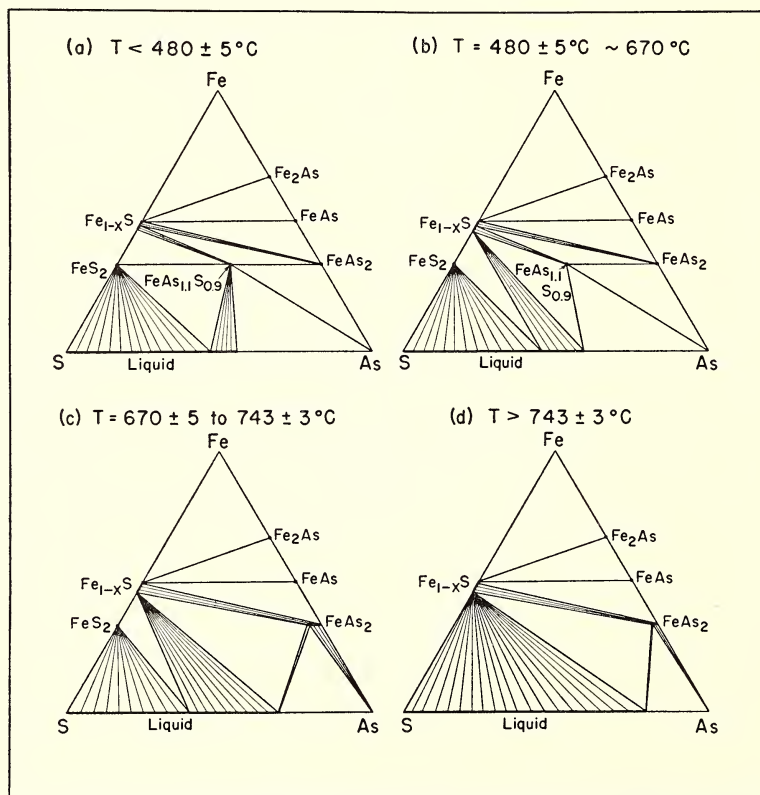


Fig. 35. Assemblages stable at various temperatures.

liquid field is too narrow to plot as more than a line in figure 34.

The eight univariant four-phase assemblages at 600° C are shown in figure 34. The limit of arsenic solid solution in iron at this temperature is approximately 3 to 7 per cent. Each boundary limit of all the four-phase regions has been determined at two places to within $\frac{1}{2}$ per cent.

At 600° C none of the binary phases can take into solid solution more than a fraction of 1 per cent of the respective ternary

composition $\text{FeAs}_{1.1}\text{S}_{0.9}$: (1) from the results of three silica tube-in-tube runs the arsenopyrite composition was calculated, the only assumption being that there is a 1:2 ratio of Fe to As+S; (2) the extensions of tie lines intersect at this approximate composition; (3) disregarding the symmetry difference in the calculation,⁸ the cell volume of synthetic arsenopyrite is

⁸ For a discussion of arsenopyrite symmetry see the Crystallography section of this report, page 246.

approximately 1 per cent larger than in the only two natural specimens measured to date, from Freiberg, East Germany, and Llallagua, Bolivia. Unfortunately, chemical analyses of these arsenopyrites are not yet available.

Arsenopyrite free of pyrrhotite inclusions could be synthesized only from bulk compositions lying within the arsenopyrite–arsenic–liquid–vapor region. The starting materials were synthetic FeAs_2 and a quenched liquid saturated with arsenic at 600°C . Near the end of a 3-week heating period at 600°C a temperature gradient of 1° or 2° along the tube was sufficient to drive the excess liquid + arsenic to one end, leaving pure arsenopyrite in the other end.

The range of stability of some of the ternary assemblages is shown in figure 35. Figure 35*b* is a slightly simplified version of figure 34. These assemblages are stable throughout a range of temperature from $480^\circ \pm 5^\circ\text{C}$ to near 670°C . The minerals pyrite and arsenopyrite, which form a common assemblage in nature, can coexist in equilibrium with each other, in the presence of a vapor, only below $480^\circ \pm 5^\circ\text{C}$, as shown in figure 35*a*. Although the temperature at which pyrite and arsenopyrite react to form pyrrhotite and liquid is subject to a pressure effect depending principally on volume change, it is unlikely to be much above 510°C within the range of geologically important pressures. The reverse reaction is extremely sluggish; it is well started but far from complete in runs heated 50 days at 450° and 470°C .

In the presence of a saturated vapor, arsenopyrite is unstable above approximately 670°C . Above this temperature, and up to $743^\circ \pm 3^\circ\text{C}$, the assemblages shown in figure 35*c* are stable. The pyrite breakdown temperature was found to be unchanged by the presence of arsenic, and it may therefore be assumed that very little arsenic is soluble in pyrite. A step intermediate between figures 35*b* and 35*c* has been omitted; the reaction $\text{FeAs}_{1.1}\text{S}_{0.9} + \text{As} \rightarrow \text{FeAs}_2 + \text{L}$ takes place at a temperature slightly lower than that of the arsenopyrite breakdown. The univariant assem-

blages arsenopyrite–loellingite–liquid–vapor, and loellingite–arsenic–liquid–vapor are then stable within a very narrow temperature range.

Above $743^\circ \pm 3^\circ\text{C}$, probably well beyond the region of geological interest, only the five univariant assemblages shown in figure 35*d* remain. FeAs and Fe_2As are stable throughout the entire temperature range, to well above 900°C . These compounds are optically very similar to loel-

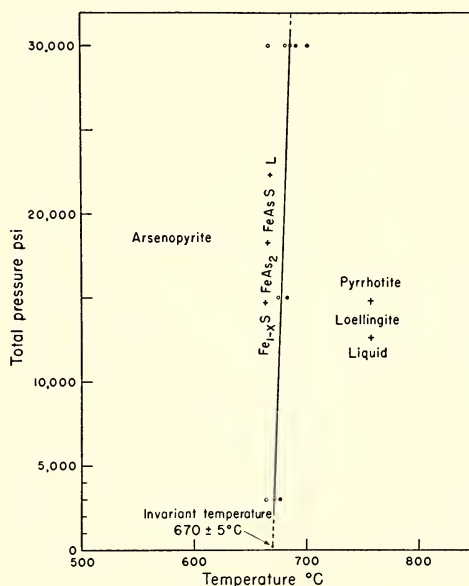


Fig. 36. Upper stability curve of arsenopyrite, $\text{FeAs}_{1.1}\text{S}_{0.9} \rightleftharpoons \text{Fe}_{1-x}\text{S} + \text{FeAs}_2 + \text{L}$.

blite and arsenopyrite. Possibly they occur in nature and have not been recognized.

Figure 36 shows the upper stability curve of arsenopyrite, determined with samples in welded collapsible gold tubes, within which, under an applied external pressure, no vapor could form. This is a univariant curve which defines the effect of pressure on the temperature at which the breakdown reaction $\text{FeAs}_{1.1}\text{S}_{0.9} \rightarrow \text{Fe}_{1-x}\text{S} + \text{FeAs}_2 + \text{L}$ can proceed, provided that no gold enters the phases. This curve, when projected down to a pressure of a few atmospheres, yields $670^\circ \pm 5^\circ\text{C}$ as the invariant temperature at which the four phases coexist with vapor. This point is

also the origin of four other univariant curves, each defining a reaction involving a vapor phase.

The upper stability curve sets a maximum temperature for the occurrence of arsenopyrite in nature, but this compound can form at any temperature below $670^{\circ} \pm 5^{\circ} \text{C}$, and its presence in nature cannot be used to demonstrate high temperature of formation, as has often been claimed. Similarly, if equilibrium between naturally occurring pyrite and arsenopyrite can be demonstrated, $480^{\circ} \pm 5^{\circ} \text{C}$ is the upper limit of formation for this mineral pair. Equilibrium assemblages formed at temperatures above 480°C may contain either, but not both, of these minerals.

THE CoAs_2 - NiAs_2 - FeAs_2 -As SYSTEM

E. H. Roseboom, Jr.

In last year's report the preliminary results of a reconnaissance in the system CoAs_2 - NiAs_2 - FeAs_2 -As at 800°C were presented. The work described here is a continuation of that study.

The arsenides in this system that occur in natural deposits are skutterudite [$(\text{Co}, \text{Ni}, \text{Fe})\text{As}_{3-x}$], the polymorphs rammelsbergite and pararammelsbergite (NiAs_2), loellingite (FeAs_2), and safflorite ($(\text{Co}, \text{Fe})\text{As}_2$). Analyses of these minerals indicate varying amounts of Co, Ni, and Fe substituting for one another. The compositions of skutterudites were discussed previously. The compositions of diarsenides will be considered below.

The diarsenides in the Co-Fe and Fe-Ni series were made by heating mixtures of Co, Ni, Fe, and As metals of the desired composition in sealed, evacuated silica glass tubes at 800°C for 60 hours. The diarsenides in the Co-Ni series were made by heating mixtures of the cobalt diarsenide phase and rammelsbergite. When native metals were used for this series, cobalt skutterudite and niccolite (NiAs) formed together with a Co-Ni diarsenide. After several regrindings and reheatings, the cobalt skutterudite and niccolite would first

diminish and then disappear, leaving the Co-Ni diarsenide. When end-member phases were used some skutterudite and niccolite formed, but they disappeared after one or two regrindings.

Figure 37 shows the cell dimensions as calculated from the 111, 210, 101, and 120 X-ray diffraction peaks in the Fe-Co, Co-Ni, and Ni-Fe diarsenides. The orientation is the revised one suggested by Buerger (1937) for minerals of the marcasite structure. The first two series form complete solid solutions. The Ni-Fe series is interrupted by a two-phase field in which a $\text{Ni}_{94}\text{Fe}_6$ diarsenide is stable with a $\text{Ni}_{70}\text{Fe}_{30}$ diarsenide. The X-ray diffraction patterns of samples with compositions lying in this two-phase field contain the peaks of both the above diarsenides, but the relative intensities vary with the composition. A sample originally heated at 800°C was divided and reheated at different temperatures. At 850°C after 7 days, the two coexisting diarsenides had d values corresponding to $\text{Ni}_{92}\text{Fe}_8$ and $\text{Ni}_{75}\text{Fe}_{25}$. At 750°C after 18 days the two diarsenides had d values corresponding to $\text{Ni}_{94}\text{Fe}_6$ and $\text{Ni}_{67}\text{Fe}_{33}$. At 700° and 600°C after $3\frac{1}{2}$ months the peaks of the iron-rich diarsenide were too weak to measure and the nickel-rich diarsenide was about $\text{Ni}_{96}\text{Fe}_4$ in both cases. Thus, there appears to be a highly asymmetrical solvus limiting the extent of solid solution in this series.

This solvus extends into the ternary compositions to at least $\text{Ni}_6\text{Fe}_1\text{Co}_1$ at 800°C because a sample of that composition produced two diarsenide phases with d values similar to those in the Ni-Fe series. A sample with equal amounts of Co, Ni, and Fe and three samples along the 70 per cent Fe, 30 per cent (Co+Ni) line gave homogeneous diarsenides.

The cobalt-rich diarsenide phase is not orthorhombic like rammelsbergite and loellingite. The 101 and 111 peaks, when traced through the solid solution series toward CoAs_2 , split into two peaks of approximately equal intensity, suggesting

that the cobalt-rich diarsenides are monoclinic.

One should be cautious about determining the composition of natural diarsenides from the curves on figure 37, because small amounts of other atoms substituting for Co, Ni, Fe, or As may affect the cell dimensions. Five unanalyzed safflorites were X-rayed, and their d values indicated com-

Other substitutions may cause changes in the cell dimensions. Neumann, Heier, and Hartley (1955), who measured the d values of several natural loellingites containing sulfur, found that increasing sulfur content causes the difference between the 120 and 101 d values and between the 210 and 111 d values to decrease. Sulfur atoms substituting for about 9 per cent of the

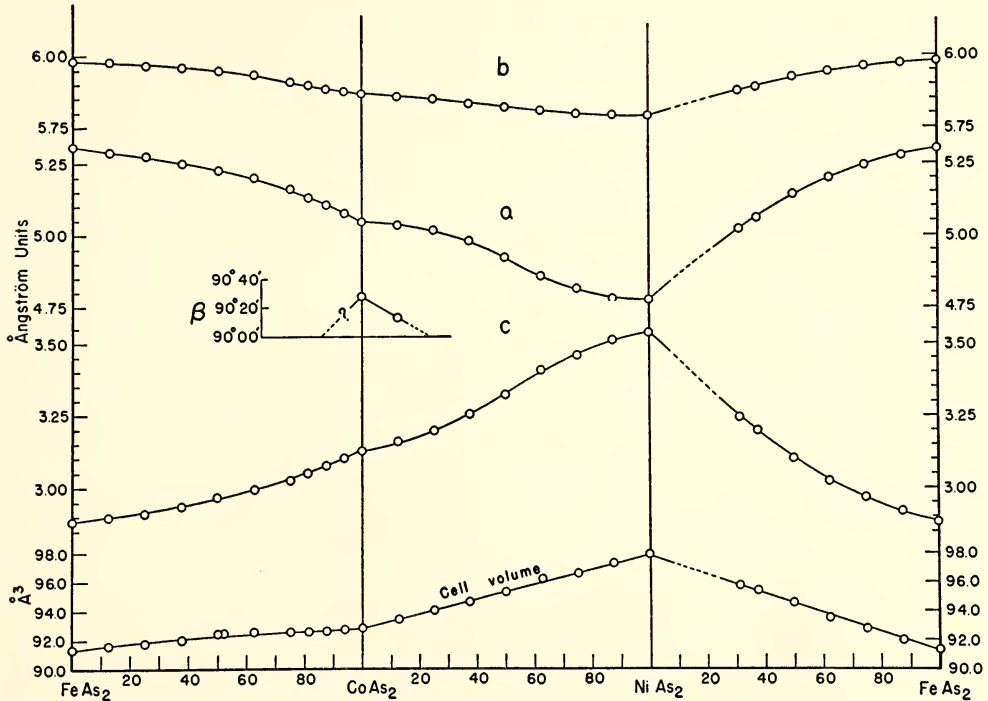


Fig. 37. The relationships between composition and cell dimensions in the diarsenides of Co, Fe, and Ni.

positions ranging from about $\text{Co}_{45}\text{Fe}_{55}$ to $\text{Co}_{85}\text{Fe}_{15}$, according to figure 37.

The experimental work revealed no systematic change in the cell dimensions with arsenic content of loellingite. The 111, 210, 101, and 120 d values of synthetic loellingites with the compositions $\text{FeAs}_{2.00}$, $\text{FeAs}_{1.99}$, $\text{FeAs}_{1.98}$, and a loellingite with a trace of FeAs in a sample with the composition $\text{FeAs}_{1.97}$ were measured, using quartz as an internal standard. The widest range of values for a single d value was 0.0019 Å, and there were no systematic changes with composition.

arsenic atoms in the most sulfur-rich loellingite resulted in a difference of 0.04 Å between the first pair of d values, and between the second pair a difference of 0.05 Å. These values for the differences correspond approximately to the substitution of 30 per cent of the Fe by Co or about 15 per cent of the Fe by Ni. Although the differences are the same in these cases, the absolute values of the d values may be different for the different substitutions. L. A. Clark is studying the sulfur content of loellingites in the system Fe-As-S.

Figure 37 also shows the cell volumes of

the Co, Ni, and Fe diarsenides. They increase in the order FeAs_2 - CoAs_2 - NiAs_2 . The change in cell volume is essentially linear. Of the unit cell dimensions, only the c axis follows this order of increasing size. The a and b axes do just the opposite, decreasing in the order FeAs_2 - CoAs_2 - NiAs_2 . Buerger (1937) and Rosenqvist (1953) have recognized two distinct divisions of compounds with the marcasite structure, a marcasite group and a loellingite group. The marcasite group has a relatively longer c axis and a larger c/b ratio than the loellingite group, suggesting a difference in the bonding between the atoms. The Ni-Co and Co-Fe series thus bridge the gap between these two orthorhombic groups with a monoclinic phase, cobalt-rich diarsenide. In the Ni-Fe series the two groups are separated by a two-phase region.

SULFIDE-WATER SYSTEMS

G. Kullerud and H. S. Yoder, Jr.

The majority of sulfide ores are generally held to have been deposited from aqueous solutions. Numerous experiments show that the solubility of the sulfides in water is very small at low temperatures and pressures. The solubility curve leads, in a continuous or discontinuous fashion, to the liquidus curve extending to the decomposition of the sulfide itself. Thus, in the case of ore deposits associated with magmas, it is conceivable that the sulfides are deposited from a concentrated solution as suggested by Spurr.

Accordingly, we have made preliminary investigations of the influence of water at high temperature and pressure on the melting points of several sulfides. It was convenient to study these sulfides in both rigid silica glass tubes and collapsible gold tubes. Runs up to 950°C and 2000 bars H_2O pressure with galena (PbS) in gold tubes failed to produce any melting. It was observed that galena at this temperature and pressure was transported through the wall of the gold tube along intergranu-

lar boundaries and deposited on the exterior. The melting point of PbS was determined as $1130^\circ \pm 5^\circ\text{C}$ (Kracek, 1952) under its own vapor pressure in silica tubes. Thus our experiments set a limit on the possible lowering of the melting of PbS under these pressures.

The melting point of acanthite, Ag_2S , was found to be $837^\circ \pm 5^\circ\text{C}$ under its own vapor pressure in silica tubes. Melting of acanthite occurred at considerably lower temperatures when the charge was held in an open gold tube inside a sealed silica glass tube, presumably because of reactions taking place between the gold tubing and Ag_2S .

Since it has been shown that gold does not measurably influence the stability relations of the Fe-S system above 300°C (Kullerud and Yoder, Year Book 55, p. 181), experiments with pyrite, FeS_2 , and water were attempted in gold tubes. The breakdown temperature of pyrite is lowered by the presence of water. Preliminary experiments indicate that the lowering is in excess of 30°C at 2000 bars H_2O pressure. Pyrrhotite and a sulfur-rich gas coexist with pyrite in its stability field. Only pyrrhotite and a sulfur-rich gas were observed when pyrite was completely decomposed.

ORE SOLUTIONS

H. L. Barnes

Theoretical. Available results of laboratory studies of mineral assemblages and their vapor pressures under anhydrous conditions can be applied thermodynamically to place useful limitations on the composition of ore solutions. Kullerud (Year Book 56) determined the phase relations in the Fe-S-O system, which contains the almost ubiquitous mineral assemblage pyrite (FeS_2), pyrrhotite (Fe_{1-x}S), and magnetite (Fe_3O_4). The common occurrence of these minerals in a variety of types of ore deposits makes conclusions based on this assemblage of widespread significance. These minerals, when coexisting in equi-

librium at any given temperature, fix the partial pressures of sulfur ($P_{S_{tot}}$) and oxygen (P_{O_2}). Kullerud and Yoder (1958) have summarized the vapor-pressure data for the pyrite-pyrrhotite assemblage. Muan (1958) has summarized the data and given T versus P_{O_2} curves for the univariant assemblages wüstite (FeO) and magnetite + vapor, iron and magnetite + vapor, and hematite (Fe_2O_3) and magnetite + vapor. These curves define, at any specific temperature, the range of P_{O_2} that can occur with magnetite as a stable phase. The direct measurement of these vapor pressures has been possible only at high temperatures, but the curves may be extrapolated below the critical temperature of water ($374^\circ C$) with sufficient accuracy for the present purpose. The vapor over the pyrite-pyrrhotite pair is predominantly sulfur vapor composed of the molecules S_2 , S_6 , S_8 , etc. Because the distribution of the molecular species is not known under these conditions, the range of possible values for P_{S_2} used in the extrapolation from the measured vapor pressures includes this uncertainty.

The minimum temperature at which the pyrite-pyrrhotite-magnetite assemblage is formed in ore deposits is not known, but ore bodies where sphalerite occurred in coexistence with this assemblage have been estimated to have formed at temperatures as low as about $200^\circ C$. The pyrite + pyrrhotite + magnetite assemblage is probably not formed much below this temperature; the partial pressures for this assemblage are shown in figures 38A, 39, and 40 only to illustrate the changes in the position of these vapor pressure fields with T and concentration.

The *ionic* character of the ore solution is a function of the five predominant variables: temperature, T ; total pressure, P_{tot} ; total concentration of sulfur-containing ions, (S_{tot}); P_{O_2} ; and pH . Bulk composition, which includes the variables (S_{tot}), P_{O_2} , and pH , is, by itself, inadequate for describing the ionic species in the aqueous

phase. Thermodynamic constants can be used to calculate the activities of major sulfur-containing aqueous ions as functions of P_{O_2} and pH if (S_{tot}) (including solids), as well as T and P_{tot} , is constant. The results of such calculations, which are of classical thermodynamic types, are illustrated graphically in figure 38. The areas shown represent regions of predominance of each of the major ionic species in the system. The lines limiting each of these areas indicate equal activities of the predominant ions of the adjacent areas. Activity equals concentration within one order of magnitude for concentrations up to about 0.1 mole/liter; therefore, for clarity, concentration will be used here instead of activity.

Although P_{tot} is taken as 1 atm at $25^\circ C$ in the calculations, inert pressures of several hundred atmospheres are not expected to change the ionic stabilities beyond the limit of error in the calculations ($<10^{0.5}$). If P_{tot} is neglected, a series of these diagrams for two or more values of (S_{tot}) at several temperatures is necessary to outline the ionic behavior in the range of interest to this study.

The effect of changing (S_{tot}) by a factor of 100 is illustrated by comparison of figures 38A and 39. Figures 38A, 40, and 41 show the change in ionic stabilities and partial pressures with increasing temperature calculated from the van 't Hoff equation. Although there are no applicable experimental data on ions in this system above $25^\circ C$, other studies (Harned and Owen, 1943) indicate that ΔH can be assumed constant as a first approximation in this equation. The error in this assumption increases with temperature, and at $250^\circ C$ in figure 41 only a rough approximation can be given of the areas of predominance of the various ions. Nevertheless, the limits placed on the partial pressures in the aqueous system by the solid phases of the Fe-S-O system in turn narrow considerably the aqueous region of interest.

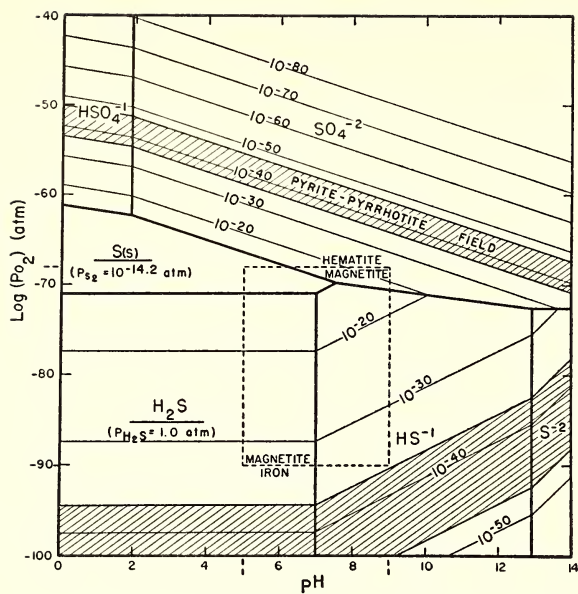


Fig. 38A

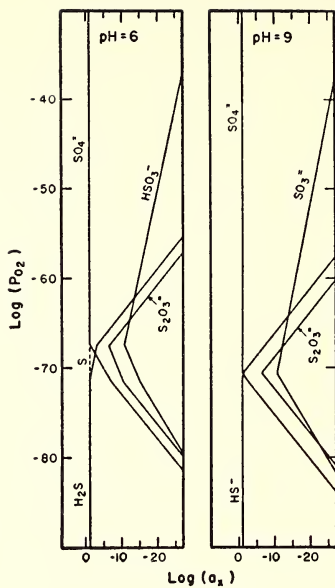


Fig. 38B

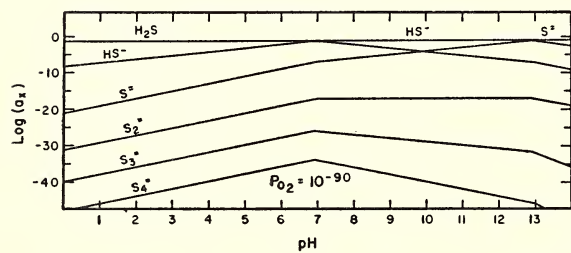


Fig. 38C

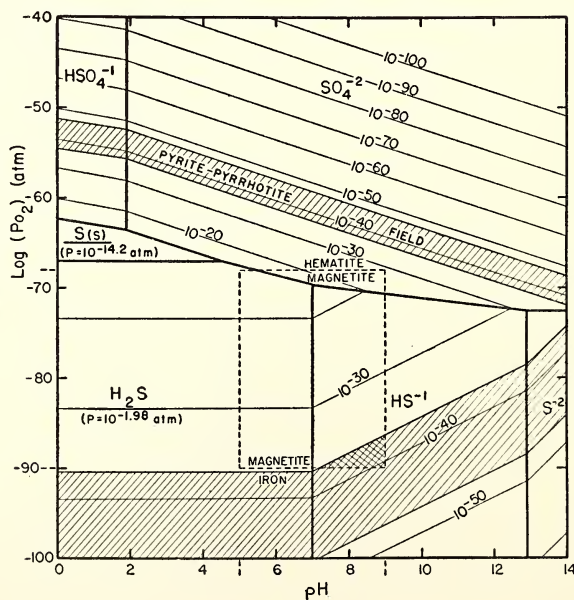


Fig. 39

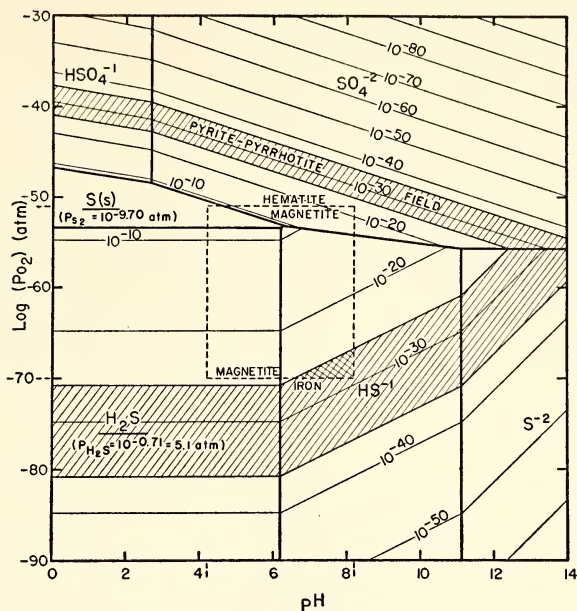


Fig. 40

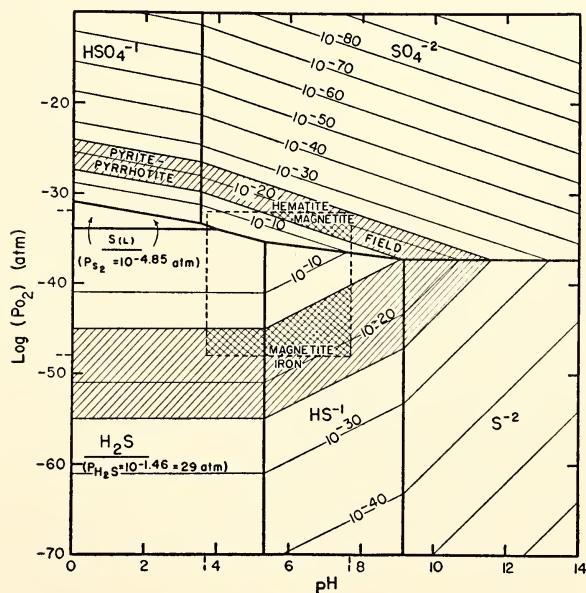


Fig. 41

Fig. 38. $S_T = 0.1$ mole/liter; $T = 25^\circ \text{C}$. A. Fields of stabilities. B. Activities of minor ions at constant pH . C. Activities of minor ions at constant P_{O_2} .

Fig. 39. $S_T = 0.001$ mole/liter; $T = 25^\circ \text{C}$.
Fig. 40. $S_T = 0.1$ mole/liter; $T = 100^\circ \text{C}$.
Fig. 41. $S_T = 0.1$ mole/liter; $T = 250^\circ \text{C}$.

Figs. 38–41. Fields of stabilities of predominant ions with partial pressures of S_2 (contoured in atmospheres) in equilibrium with water at fixed temperature and total concentration of sulfur-containing ions. See text for discussion of crosshatched areas.

The pH boundaries of this region cannot be calculated at present, but it can be assumed that during ore transport the pH will not deviate from neutral by more than 2 pH units. Variations beyond these limits are unlikely because of the buffering action of wall rocks on the ore solutions. Furthermore, measurements of the pH in pertinent hot springs, and fluid inclusions in ore minerals, lie within these limits.

The ore-transporting region shown in figures 38*A* and 39–41 corresponds to the aqueous conditions where the strongly complexing ions polysulfide ($S_x^{=}$) (fig. 38*C*) and thiosulfate ($S_2O_3^{=}$) (fig. 38*B*) reach maximum concentrations. These are the most probable complexing ions for the metals in the ore solution, as shown by independent evidence from zoning (Barnes, 1956) and by the maximum solubilities of metallic complexes in such solutions. The three lines of evidence outline an aqueous region in which the ionic character of the sulfur-containing species needs systematic experimental study.

In figures 38*A* and 39–41 the assemblage pyrite–pyrrhotite–magnetite can coexist with water at each (S_{tot}) only where the partial pressure fields overlap as shown by cross hatching. The lack of overlap of the partial pressure fields fixes an upper limit to (S_{tot}) in the aqueous phase. As seen by comparison of figures 38*A* and 39, increasing (S_{tot}) raises P_{S_2} and spreads the contours away from the sulfur field. In order to have appreciable overlap of these fields, in figure 41, (S_{tot}) *must be less than* 1.0 mole/liter at 250° C. In figure 40, (S_{tot}) must be less than 0.01 mole/liter at 100° C. An ore-transporting solution capable of depositing the assemblage pyrite–pyrrhotite–magnetite (hence in equilibrium with these minerals) cannot have a total concentration of ions containing only S, H, and O greater than the above limits at each temperature. Compounds or ions containing both metals and sulfur are excluded from this maximum.

When the stabilities and vapor pressures of other assemblages of ore minerals are accurately determined, it will be possible to ascertain the specific composition of sulfur-containing ions in the ore solution at any predetermined temperature for individual ore deposits.

Experimental. Apparatus (fig. 42) and experimental technique have been developed for measuring solubilities under the temperature and pressure conditions prevailing during the formation of many ore deposits. This apparatus is now in use in a study of the solubility of sphalerite (ZnS) in H_2S -saturated water.

The solubility in each sample in this system is determined polarographically. Preliminary results show that solubilities near 100° C and 500 psi exceed 10 mg/l ZnS, which is more than 10^6 times greater than the calculated solubility product in pure water at this temperature, but at 25° C and about 100 psi the solubility is not detectable (<1 mg/l ZnS) with the present analytical technique. The solubility is controlled by the formation of complex ions probably of the type $ZnS \cdot xH_2S$. The magnitude of the solubility indicates a surprisingly great stability for the simple sulfide complexes of zinc, which, however, are probably not predominant during ore transport. Only by a fundamental understanding of the chemistry of this relatively simple three-component system can the behavior of the four-component systems containing the complex ions, polysulfide and thiosulfate, be interpreted.

Solutions involved in ore transport are highly corrosive; therefore, the reaction vessel (internally plated with chromium), tubing, and valves are of stainless steel. Slight corrosion of the chamber has been observed up to 160° C and 1000 psi by runs in the system $ZnS-H_2S-H_2O$.

Although there are several methods of measuring solubilities at moderate pressures, the use of double valves as described below is believed to give the most quantitative results. The entire assembly shown

in figure 42 is agitated to hasten equilibrium by tilting through an arc of 30° about the horizontal rocker axis. Connections to valves 2 and 4 are made with a flexible spiral coil of tubing wound about the rocker axis.

Temperature is regulated by a bridge circuit in which the variation of the resistances of the nickel furnace windings controls the voltages supplied to furnaces *A* and *B*. The temperature is measured and recorded by thermocouples inserted in the wells shown in the figure. Pressure

after flushing the system with N_2 , a vacuum of less than 200μ is attained by pumping with only valve 4 closed. Next valve 2 is closed and the valve on the gas cylinder opened until pressure in the reaction chamber indicates a calculated gas content. The weight of gas in the apparatus is determined by reweighing the gas cylinder. With valves 2 and 3 closed, deaerated water is pumped from a weighed amount in a calibrated reservoir (flushing the gas in the sampling tube ahead) through an ion-exchange column into the

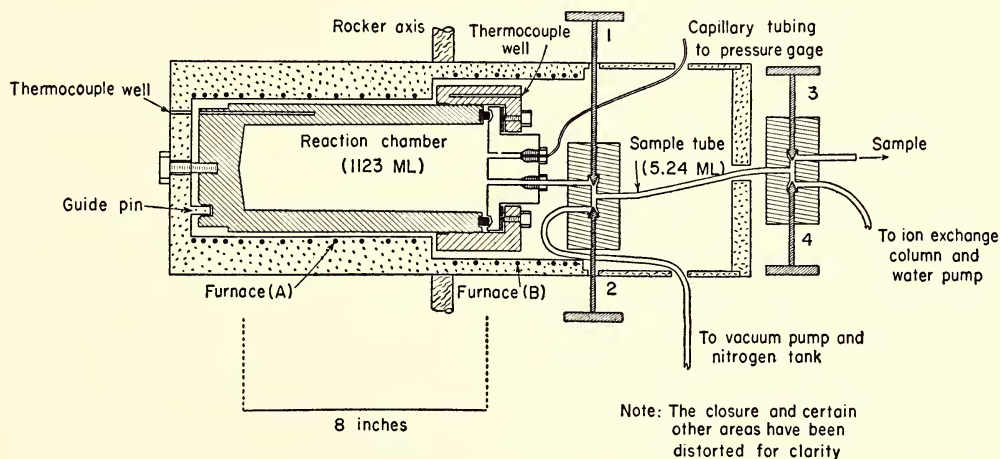


Fig. 42. Apparatus for sampling chemical systems at elevated pressure and temperature in order to determine solubilities of minerals.

is measured directly with a stainless-steel bourdon gage through a U tube filled with paraffin oil which chemically isolates the gage from the system. Paraffin oil does not react with runs in the system $ZnS-H_2S-H_2O$ if the U tube is kept near room temperature.

The procedure for each run is as follows: After thorough cleaning, the seal of the reaction vessel is tested with argon and water to avoid leaks. Without breaking the gasket seal, the chamber is emptied through the tubing ports and evacuated to <1 mm Hg to assure a dry interior. A weighed amount of solid is inserted through a tubing port, and the entire apparatus is assembled. A weighed cylinder of gas is then connected to valve 3 and,

reaction chamber to the desired volume. Valves 1 and 4 are then closed, and the water in the sampling tube is drained through valve 3 (and flushed with N_2 from valve 2) and combined with that in the reservoir to determine the weight loss and the weight of water in the reaction chamber. Thus, weighed amounts of solids, liquids, and a gas can be inserted into the reaction chamber without contamination.

After the apparatus has been brought to temperature and agitated until equilibrium is assured, a sample may be withdrawn simply by evacuating the sample tube through valve 2 with valves 1, 3, and 4 closed, opening valve 1 momentarily with valves 2, 3, and 4 closed, and then flushing

the sample through valve 3 with N_2 from valve 2. Several samples at the same or different temperatures can be taken from each charge in this manner because volume of sample is small compared with that of the main vessel.

The sample is believed to be representative because the sample tube is maintained near the same temperature as the reaction chamber and any precipitate formed in the reaction vessel near the sample tube from the instantaneous pressure drop

would be flushed into the sample as the tube fills. N_2 from valve 2 cleans any precipitate from the sample tube. The sample tube is 0.45 per cent of the volume of the reaction chamber; therefore, in the system $ZnS-H_2S-H_2O$, the observed pressure drop on sampling is ~ 0.5 per cent.

The apparatus is suitable for quantitative solubility measurements in a variety of chemical systems limited only by corrosion and the mechanical strength of the reaction vessel.

DIFFRACTION EFFECTS OF SHORT-RANGE ORDERING IN LAYERED SEQUENCES

F. Chayes

Subtle diffraction effects—peak shifts, changes of intensity, appearance or disappearance of weak reflections—are proving of great value in silicate phase-equilibria studies, particularly in the delineation of subsolidus reactions. It is becoming increasingly popular to regard such effects as consequences of ordering (or disordering). Usually no geometrical model of the ordering process is presented, and in the rare exceptions the ordering involved seems to be of the “long-range” variety, which generates additional repeats or superperiodicity along one or more of the principal directions of the crystal. The possibility that “short-range” ordering, i.e., ordering concerned with nearest-neighbor pairings rather than the development of a superlattice, might generate characteristic diffraction effects of the types in question seems to have escaped notice.

Experimental study of diffraction effects generated by varying levels of short-range ordering in layered sequences, begun late in 1956, continues as a major activity. Most of the year has been spent in refining and improving experimental techniques developed toward the close of the last report year and described briefly in Year Book 56. As often happens in such matters, the chronological order of events is determined by psychological rather than logical considerations, and makes little sense to any-

one but the investigator himself. The year's progress is accordingly reviewed here in rational rather than chronological sequence, treating, in order, developments affecting the design of random-layered sequences characterized by some arbitrary level of short-range order, the production of experimental diffraction masks based on such sequences, the generation of diffraction transforms from these masks, and the direct calculation of the diffraction effects by high-speed computation.

The Distribution of Run Lengths

The number of unbroken runs of identical elements in a two-element sequence such as $ABBBAAAB \cdots$ determines the level of short-range order. For any possible number, d , of runs, it may be shown that the probability that any run chosen at random is of length i is:

$$q(a)_i = \binom{N_A - i - 1}{d - 2} \div \binom{N_A - 1}{d - 1} \quad (1a)$$

or

$$q(b)_i = \binom{N_B - i - 1}{d - 2} \div \binom{N_B - 1}{d - 1} \quad (1b)$$

where the array contains N_A A's and N_B B's. Since there are to be d runs of each element, and the probabilities $q(a)_i$ and $q(b)_i$ are the same for each run of the appropriate element, the expected number

of runs of length i for each element is simply the product of the total number of runs of the element by the probability that any one of these will be of length i , or

$$E(d_{Ai}) = dq(a)_i \quad (2a)$$

$$E(d_{Bi}) = dq(b)_i \quad (2b)$$

The importance of these relations is that they permit the construction of experimental—and therefore necessarily rather short—sequences having any desired level of ordering and at the same time exhibiting relative frequencies of run lengths as close as possible to those required by theory. The distribution of run lengths is thus no longer an experimental variable. The sequence in which the runs of different lengths occur in an actual mask is randomized by a standard Monte Carlo sampling technique, but, for a mask of any given length, composition, and level of ordering, this is the only random variable. This is essentially the situation that confronts us when we examine the diffraction pattern of a real crystal in which the level of ordering is not subject to change during the time necessary to photograph the pattern.

Generation of Diffraction Masks of Layered Sequences

In the two-dimensional mask, the place of a layer is taken by a row of equally spaced holes in an opaque matrix. In the work reported last year, these were punched individually on a small instrument, the Peek-a-boo Punch, designed by the Basic Instrumentation Laboratory of the National Bureau of Standards. The masks were of excellent quality, but sequences longer than 150 layers could not be managed, and even a 100-layer mask usually required more than 3 hours of machine time.

The instrument now used for this purpose, shown in figure 43, is an adaptation of one described by B. G. M. Willis in 1957. It is essentially a contact printer, in which the photographic element, an 8 by

10 inch plate, may be displaced laterally between exposures.

On the stage of a large dividing engine (A), a smaller one (B) is mounted, the threads of the two being normal to each other. The stage of the smaller engine carries a trough (C) in which the plateholder (D) rides. The light box (E) is mounted rigidly to the frame of the larger dividing engine, and from it a large light shield (F) is spring-loaded against the top of the track that carries the plateholder. From inside the light box a rectangular shoe containing the template is spring-loaded, through a recess in the light shield, against the plate cover. Two 100-watt projection bulbs mounted in the removable top of the light box (G) are activated by the switch of an exposure timer (H).

The loaded plateholder is slid into its track; the shoe containing the template is next lowered through the light shield and brought to rest squarely on the plate cover. The cover of the light box is put in place, making the entire assembly light-tight. Finally the plate cover is withdrawn, and the template, which is resting on it, drops gently onto the plate.

The template now in use is simply a brass strip containing a row of 60 holes of 0.5-mm diameter spaced 3 mm apart, parallel to the screw of the upper dividing engine. Each exposure thus generates a row of "scatterers" which serves as a two-dimensional projection, or model, of a layer in a layered structure. The distance between layers is obtained by translation of the plate with the large dividing engine; this interval, being constant in the problem now under consideration, is established by means of a detent, so that the large drum need be read only as a check. Each exposure is followed by a rotation of the large drum; the cross engine, however, is reset only when two successive layers are separated by an offset or "mistake." After a little practice, a mask of 200 layers can be completed in a little less than an hour. If of suitable quality, the original pattern is reduced by a linear factor of not less than 8. If an eightfold reduction is used, the

distance between layers (rows) in the finished mask is 0.125 mm and the viewing assembly of the diffractometer described below will contain two full orders of the diffraction pattern. The photographic work has so far been done entirely on glass Kodak plates for the original and high-contrast Eastman lantern slides for the reductions. A typical mask is shown in figure 45a.

The Optical Diffractometer

The very small diffractometer used to obtain the transforms illustrating last year's report has now been replaced by the instrument shown in figure 44a; figure 44b is a schematic diagram. The image contained in the viewing assembly is remarkably sharp and clear. Photographs of equal clarity can be obtained with early versions of the 2-watt concentrated (Zr) arc lamp as light source. The currently available bulbs of this type are subject to considerable flicker, and, since exposure time is of the order of 1 or 2 minutes, the photographs are commonly rather inferior. The difficulty is much reduced by use of a pinhole, but this greatly increases exposure time.

A good summary of the impact of these developments on the experimental work may be obtained by comparing figure 45b with figure 4b (plate 2, p. 192) of Year Book 56. The composition and level of ordering of the masks yielding these transforms are the same.

The Calculation of Intensity Profiles

The number of (atomic) layers in a crystal of a layered mineral is ordinarily very large, and a considerable increase in mask length would therefore be desirable. With the procedure reviewed above, it is possible to design sequences of any length, composition, and level of ordering. Although in principle the instrument described above could be made to generate very long masks, the practical maximum seems to be of the order of 400. And, if a mask of even this length is reduced suf-

ficiently to permit complete illumination in the central region of the collimated beam of the diffractometer, the viewing assembly will not contain a full order of the diffraction pattern. The desired extension of mask length thus requires either rather fundamental improvements in optical equipment or an outright transfer of the work from optical to numerical experimentation. Ultimately, it may be useful to explore both possibilities, but at the moment full attention is being given the latter.

In a mask like figure 45a all the rows are of identical scattering power, and each row is either in register with the first row or offset from it by a fixed proportion (t) of the distance between points. In the experimental work described here $t = \frac{1}{2}$ and the x coordinate of the first hole in each row is thus 0 or $\frac{1}{2}$. The y coordinate is $(n-1)/N$, where n is the number of the row and N the number of rows in the mask. The intensity of the diffracted beam of order (hk) is then given by the Fourier transform

$$I_{hk} = \left(\sum_1^N e^{2\pi i(hx_n + ky_n)} \right)^2 \\ = \left[\sum_1^N \sin 2\pi(hx_n + ky_n) \right]^2 \\ + \left[\sum_1^N \cos 2\pi(hx_n + ky_n) \right]^2 \quad (3)$$

It is implicit in the definitions of sine and cosine that, for any h , $I_{hk} = I_{h(N+k)}$; i.e., the transform is periodic along k with a repeat of N . It may also be shown that, for $0 < k$ (integral) $< N$,

$$I_{hk} - I_{h(N-k)} = 4 \sin 2\pi ht \cdot \\ \left[\sum_1^N \cos B_t(n) \sum_1^N B_0(n) - \sum_1^N \sin B_t(n) \cdot \right. \\ \left. \sum_1^N \cos B_0(n) \right] = 0 \quad (4)^9$$

⁹ Derivation of equations 4 and 5 is straightforward but tedious. Details will be presented in journal publication.

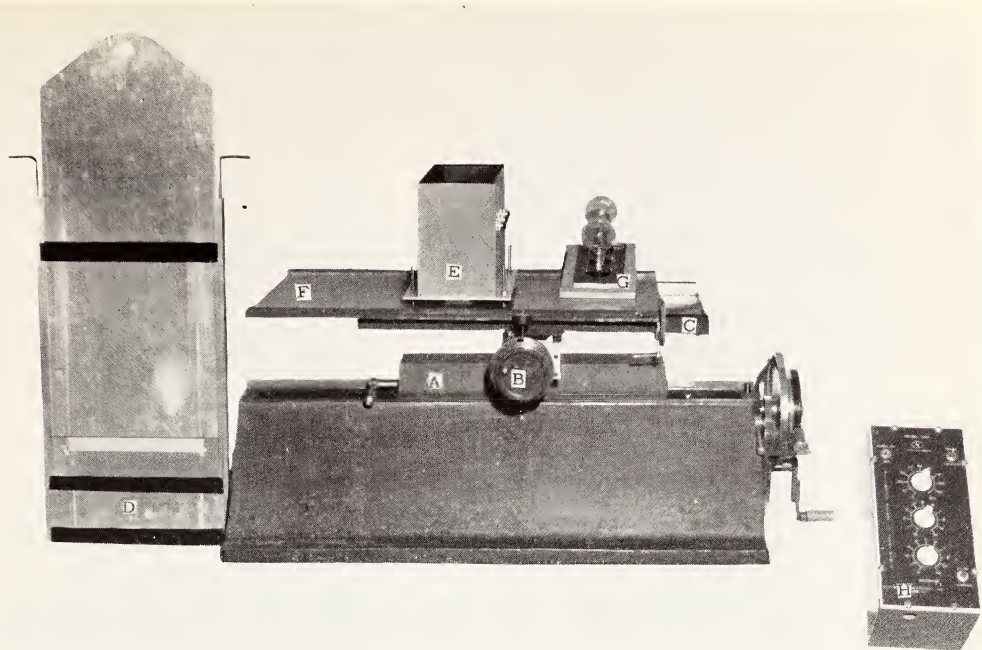
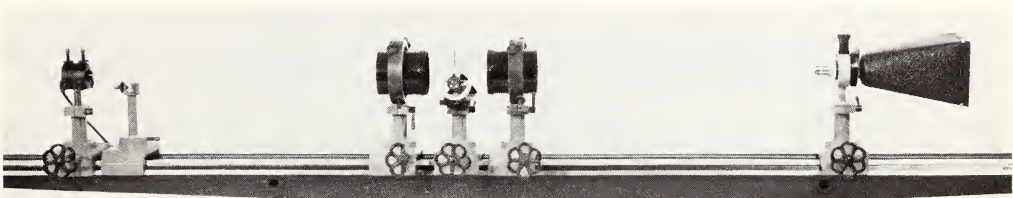
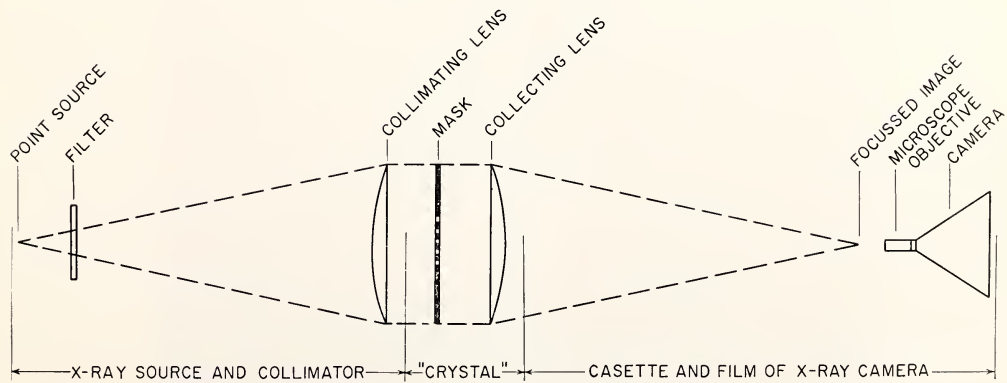


Fig. 43. Mask generator. For explanation of letters see text.



(a)

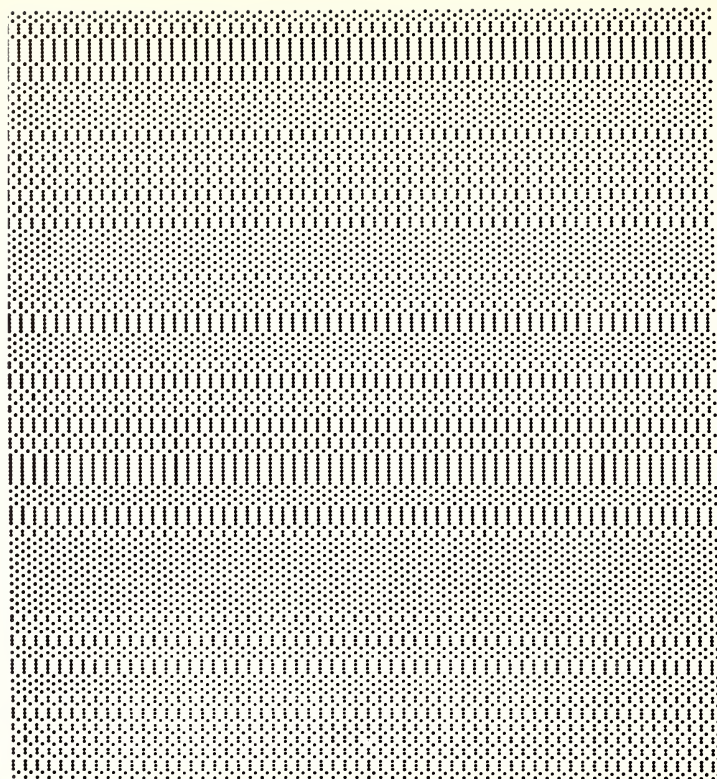
OPTICAL COMPONENTS



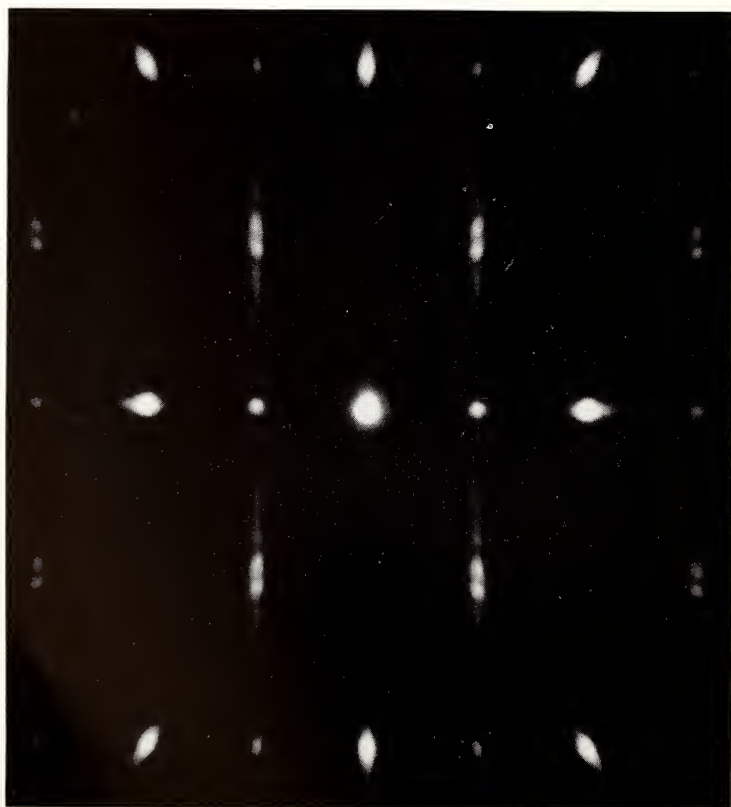
MODEL EQUIVALENTS

(b)

Fig. 44. Optical diffractometer. (a) Photograph of the instrument. (Focal length of lenses is 45 cm.) (b) Schematic diagram identifying components.



(a)



(b)

Fig. 45. (a) A short-range ordered 2:1 sequence, $N = 192$. (b) Transform of (a). (Compare with fig. 46, plate 2, Year Book 56.)

where $B(n) = (2\pi k/N)(n-1)$, the summations to be carried through in each case only for the layers having the x value indicated by subscript.

Finally, it may be shown that, for k integral, equation 3 may be written

$$I_{hk} = 2(1 - \cos 2\pi ht) \left(\left[\sum_1^N \cos B_0(n) \right]^2 + \left[\sum_1^N \sin B_0(n) \right]^2 \right) \quad (5)^9$$

in which form it is apparent that if $t = \frac{1}{2}$, as in the experimental work here described, I_{hk} will be the same for all h odd and 0 for all h even.

The calculations which are to evaluate

the influence of short-range ordering on "diffuse" scatter may thus consist merely of the computation of I_{hk} in the region $0 < k < N/2$ (instead of $0 < k < N$, as in equation 3) and each I_{hk} value will require the generation of only N_0 —or N_t —sines and cosines (instead of N , as in equation 3).⁹ At the present writing the trial and testing stage of the programming is nearing completion, but systematic computation has not begun.

Perhaps the best summary of this report is to say that the year has been spent preparing to examine, and perhaps to solve, some of the problems posed by the work reported last year. The preparation is about completed.

CRYSTALLOGRAPHY

NEUTRON DIFFRACTION STUDIES

Magnetic Structure of Chalcopyrite

G. Donnay et al.

On December 16, 1957, the California Institute of Technology celebrated the fortieth anniversary of the publication of the first crystal structure to have been determined in the United States, that of chalcopyrite, CuFeS_2 . (Contribution No. 3 from the Chemical Laboratories of the Throop College of Technology.) It seems appropriate, at this time, to recall that this pioneering work was made possible by a grant of the Carnegie Institution of Washington to A. A. Noyes, at whose suggestion the research was carried out. The support which the Institution has given to X-ray crystallography and the interest it has manifested in the results thus go back to the very beginning of this science in our country.

Although the original structure of chalcopyrite was later modified and carefully refined by Pauling and Brockway (1932) on a crystal from Joplin, Missouri, it was still claimed to be the correct one by Kôzu and Takané (1934), who used a crystal from the Arakawa Mine, Ugo province, Japan. The uncertainty that was thus created was of much concern to Drs. Corliss,

Hastings, and Elliott at the Brookhaven National Laboratory. They found, by magnetic susceptibility measurements, that chalcopyrite is antiferromagnetic up to 200° C, the highest temperature at which measurements were made. They had already obtained powder neutron diffraction data on material from the locality in Japan. To interpret these data they had to be sure of the crystal structure. G. Donnay (together with J. D. H. Donnay) joined this group of workers at Brookhaven for the summer of 1957. To rule out the possibility that the material from Japan was a quenched high-temperature form of chalcopyrite, single-crystal X-ray studies were made on specimens from both localities. The outstanding difference between the two proposed crystal structures is that the Kôzu-Takané structure is tetragonal but metrically pseudocubic, $c/a = 0.983$, whereas the Pauling-Brockway structure is tetragonal with a c/a ratio equal to 1.97. Rotation patterns about the c axis, taken with cobalt radiation so as to enhance the difference in scattering powers of copper and iron, confirm the Pauling-Brockway structure for both localities; thus, up to now, only one form of chalcopyrite has been found in nature.

Single-crystal neutron data were then collected on specimens from both localities. These data can be accounted for as follows: The electron spins are taken to be parallel and antiparallel to the c axis, the moments being equal in magnitude for atoms of the same species; the copper moments must be set equal to zero (or at any rate less than 0.02), and the contribution of the aligned iron moments must equal 3.85 Bohr magnetons per atom. With this spin arrangement the space group $I4_2d$ of the Pauling-Brockway structure is preserved. Equally good agreement could be obtained with a somewhat more complicated model in which the moments of the iron atoms are of two kinds, slightly different in magnitude, and the difference between them is fortuitously compensated by small unequal moments on the copper atoms; this model, however, requires the "chemical" space group to be abandoned. In either case the agreement between observed and calculated intensities is excellent; the residual

$$R = \Sigma ||F_o| - |F_c|| / \Sigma |F_o| \text{ is } 0.04$$

for the combined data from the two localities.

The first hypothesis (copper moments equal to zero) is preferred because it requires the material to be antiferromagnetic without having to depend on fortuitous numerical values for the moments. This hypothesis implies that the chalcopyrite formula should be written $\text{Cu}^+\text{Fe}^{+++}\text{S}_2$, which in turn would lead to the prediction that 5 Bohr magnetons should be associated with each iron atom. If iron is assumed to form an additional bond with sulfur, an assumption supported by the fact that the Fe-S distance is shorter than the Cu-S distance by 0.12 Å, the reduction of the iron moment from 5 to 3.85 Bohr magnetons can be accounted for.

Symmetry of Magnetic Structures

G. Donnay et al.

In crystal-structure work, atoms are implicitly assumed to have complete spherical

symmetry, so that they can occupy any site in any space group. The point-group symmetry of the site, which results from the neighboring arrangement of matter, can always be conferred upon the atom that occupies it. In magnetic-structure studies, however, the atom that carries a magnetic moment has its own symmetry lowered to that of an axial vector, that is ∞/m . It can be visualized as the symmetry of a circular loop in which a current circulates in a given sense. Some space-group sites are, therefore, forbidden to magnetic atoms; only if the point-group symmetry of the site is a subgroup of ∞/m can a magnetic atom be placed on it. Such a site possesses at most one symmetry direction; the magnetic moment must be directed along it.

In the early days of crystal-structure determinations, no use was made of space-group symmetry; each crystal structure, considered to be a completely new problem, was solved by a trial-and-error procedure requiring considerable intuition. Nishikawa's suggestion to use space-group criteria systematically in order to limit the number of trial structures met considerable skepticism at first. This skepticism is indeed difficult to appreciate nowadays when the International Tables are universally employed by all workers in X-ray structure determination.

We should now like to repeat Nishikawa's appeal, addressing it this time to neutron-diffraction workers who are determining magnetic structures. They are at present using symmetry considerations only to obtain the chemical structure. In the next and most interesting step of their structure determination, namely finding the directions of the axial vectors that represent the magnetic moments on the various magnetic atoms, they pay no attention to the restrictions imposed on the vector directions by the presence of symmetry elements. The argument usually given to defend this approach is the following: the symmetry is not lowered when the substance becomes magnetic; the axial vec-

tors alone may eliminate some or all symmetry elements of the chemical space group without causing sufficient changes in atomic parameters to make the lowering of symmetry detectable by X rays. But this reasoning is basically no more valid for magnetic structures than for chemical structures, which may all turn out to be triclinic too if we wish to take a pessimistic point of view. There is only one difference: in dealing with magnetic moments we must keep in mind that antisymmetry elements are as likely to occur as symmetry elements.

If, then, an optimistic approach, analogous to that used in chemical-structure determinations, is taken, we shall first make use of the magnetic extinction criteria to determine the magnetic diffraction aspect which will lead to one or more magnetic space groups. If one of these magnetic space groups is the same as the chemical space group, it will be tested first. This will, in general, lead to a very small number of trial structures. If none of them yields satisfactory agreement between calculated and observed structure factors, we next try the isomorphous space groups in which some or all of the symmetry elements are replaced by antisymmetry elements. (These space groups are among the 1191 Shubnikov groups listed by Belov et al.) None of these space groups introduces additional atomic parameters.

Indeed, the justification for the proposed procedure is the desirability of minimizing the number of parameters to be determined: it does not appear justifiable to assume that the atomic parameters remain, say $1/4, 1/4, 1/4$, in a triclinic structure, where they should be given as $0.25 \pm \Delta x, 0.25 \pm \Delta y, 0.25 \pm \Delta z$. Finally, if none of the antigroups leads to agreement, a space group with lower symmetry must be tried, and the number of atomic parameters must be increased. Eventually a triclinic space group may thus be reached although to our knowledge no such case is on record as yet.

Of the two kinds of reflection, nuclear

and magnetic, that may contribute to a neutron-diffraction peak, let us consider the magnetic one only. Its sign depends on the sense of the moment, which may be reversed by some of the symmetry operations in the space group, leading to a reversal of some of the extinction rules. For this reason the appearance of additional peaks on a neutron-diffraction pattern (as compared with the X-ray pattern) indicates antiferromagnetism and can be used to determine the magnetic diffraction aspect and associated space group(s).

The example of chalcopyrite (see above) is a case in point. Its space group $I\bar{4}2d$, determined by X rays by Pauling and Brockway, forbids all reflections HHL for which the sum of the indices is not a multiple of 4. This rule holds for nuclear contributions. Magnetic reflections, however, obey the reversed rule; the fact that reflections 110, 114, 222 (with sum even but not divisible by 4) are observed is thus a confirmation of the existence of the d glide planes in the magnetic structure.

An example of the change of symmetry from the "chemical" to the magnetic space group is provided by Cr_2O_3 . The structure is known; the space group determined by X rays is $R\bar{3}c$, and the four chromium atoms in the rhombohedral cell lie on the threefold axis, in position $4c$ of multiplicity 4. Brockhouse (1953) found Cr_2O_3 to be antiferromagnetic and determined the spin arrangement from neutron-diffraction data. Pairs of chromium atoms related by a center of symmetry in the chemical space group carry compensating magnetic moments.

Symmetry considerations immediately give us information about the spin direction: the spins must be directed along the threefold axis if the magnetic structure is based on a rhombohedral lattice. But we know that the operation of inversion leaves the direction of an axial vector unchanged. It follows that the chemical space group $R\bar{3}c$ is incompatible with the spin arrangement determined by Brockhouse. The antigroup in which the centers of sym-

metry are replaced by anticensers is $R\bar{3}'c'$ (No. 106 in the listing of Belov et al., 1957). This group correctly describes the magnetic structure of Cr_2O_3 . To our knowledge this is the first magnetic structure shown to belong to a space group of antisymmetry.

SULFIDES

High-Temperature Chalcopyrite

G. Donnay and G. Kullerød

Only one form of chalcopyrite has been found in nature; it is tetragonal, $a=5.24$ to 5.32 Å, $c=10.34$ to 10.45 Å (see above, under Magnetic Structure of Chalcopyrite). The existence of a cubic, high-temperature modification has now been established. It has been obtained in the laboratory in the form of single crystals (0.05 mm in largest dimension), when a sample of pure CuFeS_2 was held at 600°C over a period of 21 months and then quenched in water. To confirm the cubic symmetry, crystals were examined with $\text{Co K}\alpha$ radiation on precession, Weissenberg, and rotation cameras. The diffraction aspect is F^{***} . The value of the cell edge was refined by a least-squares analysis of powder data obtained on the Norelco diffractometer: $a=5.264\pm0.003$ Å.

Although the crystal structure of the high-temperature form has not yet been analyzed in detail, a preliminary study indicates that it is isostructural with sphalerite, ZnS , in which case Cu and Fe atoms must randomly replace Zn atoms. The cell edge of ZnS , $a=5.406\pm0.005$ Å, differs from that of cubic CuFeS_2 by only 2.8 per cent, so that a complete series of substitution solid solutions would be expected at high temperature. This is not observed; 10 per cent (by weight) of CuFeS_2 in ZnS at 600°C exceeds the solubility limit. The saturated $(\text{Zn,Cu,Fe})\text{S}$ composition has $a=5.410\pm0.005$ Å, equal within experimental error to the cell edge of the pure end member. Similarly, 10 per cent (by weight) of ZnS in CuFeS_2 at 600°C exceeds the solubility limit. Again the cell

edge does not change measurably on maximum substitution of Zn for Cu and Fe.

Arsenopyrite

N. Morimoto

Arsenopyrite is one of the common sulfide minerals. Buerger has studied the structure of the arsenopyrite group in detail (1936, 1937). According to him, arsenopyrite usually shows twinning, and it is very difficult to find single crystals in nature. Using the data of twinned crystals of arsenopyrite combined with his knowledge of the gudmundite (FeSbS) structure, he obtained a marcasite-type structure for arsenopyrite.¹⁰

In an attempt to clarify the mechanism of twinning in arsenopyrite, to determine its structure more precisely, and to study possible structural changes with changing composition and temperature of formation, the examination of natural and synthetic arsenopyrite was undertaken in cooperation with L. A. Clark. The following summary shows some of the results obtained so far by X-ray diffraction methods.

Natural arsenopyrite. Arsenopyrite from Freiberg, Germany, was studied. Spectrographic analysis revealed less than 0.2 per cent total impurities in this material with Fe, As, and S as the only major constituents.

The cell dimensions obtained from powder patterns taken on the Philips diffractometer are $a=5.744$ Å, $b=5.676$, $c=5.784$, and $\beta=112^\circ 10'$ (by L. A. Clark). Metrically the lattice is monoclinic. The pre-

¹⁰ Buerger (1939) uses an unconventional monoclinic setting, in which the positive senses of the c and a axes enclose an acute angle β . To obtain the *conventional monoclinic setting* (β obtuse) from the Buerger setting (1939), we must apply the transformation: $\bar{1}00/0\bar{1}0/001$. To obtain the orthorhombic B setting from the monoclinic P setting, Buerger's transformation is $101/010/\bar{1}01$; the transformation $\bar{1}01/0\bar{1}0/101$ gives Buerger's orthorhombic B cell from the conventional monoclinic P cell. (To keep the sense of b , the transformation $101/010/101$ might have been preferable.) The Buerger B cell will be retained to facilitate comparisons.

cession and Weissenberg photographs of five specimens of this material always gave a triclinic symmetry of intensity distribution and a splitting of reflections. This suggests that the real symmetry of this arsenopyrite is triclinic and that twinning always exists.

Some of the specimens gave photographs that clearly show the splitting of reflections, with twin plane $(10\bar{1})$ and twin axis $[10\bar{1}]$. This splitting is easily observed, owing to the difference in length of the a^* and c^* axes. An arsenopyrite crystal heated at 600°C for 4 days in a sealed evacuated silica glass tube shows different twinning, with twin plane (101) and twin axis $[101]$. This specimen, however, had not been studied before heating.

Of five unheated specimens examined, two did not show any of the above-mentioned twinning; however, they all gave photographs on which the $(h00)$, $(0k0)$, and $(00l)$ reflections were split into two parts and the (hkl) reflections into three or four parts. Usually the splitting was very slight and the individual reflections were joined by diffuse intensity streaks. The multiple splitting is best explained by the assumption that albite and pericline twinning are present jointly as in microcline (Laves, 1950).

Therefore, this natural arsenopyrite contains two types of twinning. The combination of albite and pericline twinning which takes place as the result of the pseudo-monoclinic symmetry of the mineral is so fine that it is impossible to obtain a truly single crystal. The second type of twinning is due to the pseudo-orthorhombic symmetry and has twin planes (101) or $(10\bar{1})$. As the scale of this twinning is rather large compared with the former type, we can occasionally obtain an edifice which shows only the small-scale type of twinning.

The existence of a symmetry center was postulated on the basis of a Wilson test on the intensity distribution of the $(hk0)$ reflections. The space group of the natural arsenopyrite is accordingly $C_i\text{-}P\bar{1}$, with

some structural extinctions. An accurate structure analysis is in progress based on $(h0l)$, $(0kl)$, and $(hk0)$ reflections. The structure determined by Buerger is fundamentally correct, except that it was based on space group $C_{2h}^5\text{-}P2_1/c$.

Synthetic arsenopyrite. The only "single crystal" examined so far was synthesized at 350°C by L. A. Clark from a bulk composition lying in the arsenopyrite-pyrrotite-liquid-vapor four-phase region of the Fe-As-S system. The composition of the arsenopyrite is near $\text{FeAs}_{1.1}\text{S}_{0.9}$.

The cell dimensions determined from the powder patterns (by L. A. Clark) are $a_m = c_m = 5.796\text{ \AA}$, $b_m = 5.720\text{ \AA}$, and $\beta = 113^\circ 21'$. As the length of the a axis is the same as that of the c axis, it is possible to calculate the dimensions of the orthorhombic cell in which $a_o = 9.661\text{ \AA}$, $b_o = 5.710\text{ \AA}$, and $c_o = 6.403\text{ \AA}$.

The "single-crystal" precession and Weissenberg photographs also give orthorhombic symmetry of intensity distribution, with values of $a_o = 9.669\text{ \AA}$, $b_o = 5.710\text{ \AA}$, and $c_o = 6.400\text{ \AA}$ (all ± 0.3 per cent). Examination of all reflections observed reveals that (hkl) appears only when $h+l=2n$ and $(0k0)$ only when $k=2n$. If we accept the existence of a symmetry center, the only possible space group is $D_{2h}^{19}\text{-}Bmmm$ and structural extinctions occur for $(0k0)$, k odd. Further examination also gives the additional rules: (1) $(0kl)$ appears only when $k+l/2=2n$, and (2) $(h0l)$ only when $h+l=4n$ or $h-l=4n$.

To account for space group $Bmmm$, we must (1) assume disorder of As and S in the structure, or (2) postulate minute twinning of a monoclinic crystal, or (3) discard the marcasite-type structure. This structure, however, gives fairly good agreement between observed and calculated F values in natural arsenopyrite. Taking into account the additional extinction rules, the second assumption seems to be the most reasonable.

If we assume that the apparent orthorhombic symmetry in the synthetic arsenopyrite is due to twinning of a monoclinic

crystal (cell dimensions of L. A. Clark, above) by reflection on the (101) plane, the transformed extinction rules lead to space group $C_{2h}^5-P2_1/c$. The twinning of this space group explains the second additional extinction rule noted in the orthorhombic cell.

The accurate structure analysis of synthetic arsenopyrite is hampered by the fact that it has been impossible to get a crystal free from twinning. Nevertheless, the analysis is in progress, using reflections that are not affected by twinning. The cell dimensions determined from powder patterns of various samples of synthetic arsenopyrite are constant, and for all samples the composition appears to be close to $FeAs_{1.1}S_{0.9}$ (see report of L. A. Clark). For natural arsenopyrites, however, the cell dimensions differ measurably from locality to locality, suggesting that their compositions deviate from that of the synthetic material.

Bornite

G. Donnay, J. D. H. Donnay, and G. Kullerud

Bornite, Cu_5FeS_4 , although a common and widespread sulfide mineral, has rarely been reported in single crystals. Well formed, steel-blue, euhedral crystals were found by Kullerud at Copper Corp. Mine, Ontario. The hand specimen shows them occurring with barite crystals, an association previously reported only from the Cheshire locality. The dominant form of the bornite crystals is the cube, truncated by small octahedral faces. Their cube edge ranges in length from 0.2 to about 1.5 mm. All cube edges are replaced by staircase-like indentations, consisting of an alternation of two faces of the octahedron; for instance, the three cube edges that meet at the corner truncated by (111) are replaced by an alternation of (111) faces with ($\bar{1}\bar{1}\bar{1}$), ($\bar{1}\bar{1}1$), or ($1\bar{1}\bar{1}$), respectively, according as the edge is parallel to the *a*, *b*, or *c* axis. The height of these steps ranges from 0.01 to 0.5 mm.

Several chips were examined on the rotation, Weissenberg, and precession cameras

using Mo $K\alpha$, Cu $K\alpha$, and Co $K\alpha$ radiations. Two types of bornite were observed: one is cubic, diffraction aspect Fd^{**} , space group $Fd\bar{3}m$ if holohedral, cell edge 21.94 ± 0.06 Å; the other has a primitive orthorhombic lattice, with pseudotetragonal cell dimensions, $a=b=21.90 \pm 0.006$, $c=10.95 \pm 0.03$ Å. The latter type has previously been reported by Frueh (1950) on low-temperature crystals from Bristol, Connecticut. In addition Tunell and Adams (1949) have reported a cubic type with cell edge 32.8 Å. The original structure determination by Lundquist and Westgren (1936) assumed a cubic cell, 10.93 Å along the edge. (It is not certain, however, whether weak reflections were taken into account or ignored in this study.) Thus we now know of at least three types of low-temperature bornite, two of which have been found on the same hand specimen and appear to belong to a single generation.

Like many other sulfides, bornite has a crystal structure composed of sulfur atoms in a face-centered cubic arrangement, cube edge 5.47₅ Å, and of metal atoms that must be distributed in the available holes: at $1/4$ $1/4$ $1/4$, etc., in tetrahedral coordination, at $1/3$ $1/3$ $1/3$, etc., in trigonal coordination, and at $1/2$ $1/2$ $1/2$ in octahedral coordination. The various low-temperature forms must differ in the arrangement of the metal atoms. There is good evidence (Frueh, 1950) that, in the high-temperature form (cubic, with cell edge 10.93 Å), copper, iron, and vacant sites form a substitution solid solution; in other words, disorder exists among cations both as to chemical nature and as to atomic position.

The large cell contents of the various bornite types—1080 copper atoms, 216 iron atoms, and 864 sulfur atoms in the large cube described by Tunell and Adams—make us suspect that we are dealing with a complex edifice, either a twin as in digenite (Donnay, Donnay, and Kullerud, 1958) or a syntactic intergrowth of two distinct phases as in andorites IV and VI

(Donnay and Donnay, 1954). The latter hypothesis accounts for the specimens that show a cube edge of $32.8 = 6 \times 5.47 \text{ \AA}$ and have their first, fifth, seventh, and eleventh layer lines completely absent on the a -axis rotation photographs presented by Tunell and Adams. The absences are accounted for if we postulate a syntactic intergrowth of two cubic phases, a known one with cell edge $10.94 = 2 \times 5.47 \text{ \AA}$ and a hypothetical one with cell edge $16.41 = 3 \times 5.47 \text{ \AA}$ (fig. 46). Unfortunately, however, we

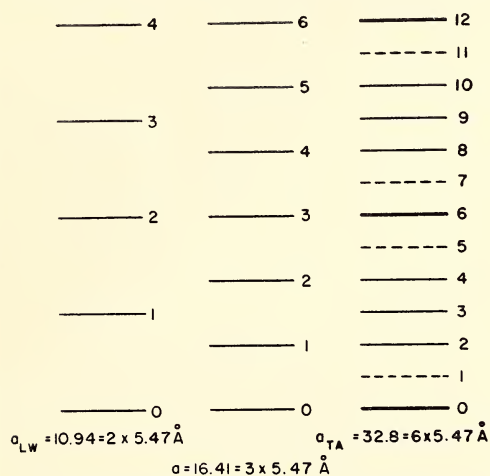


Fig. 46. Schematic explanation of bornite rotation pattern (observed by Tunell and Adams). The proposed intergrowth of two cubic forms, one with $a = 2.547 \text{ \AA}$ (found by Lundquist and Westgren) and a second one with $a = 3.547 \text{ \AA}$ (not yet reported in the literature) accounts for the observed absence of first, fifth, seventh, and eleventh layer lines.

cannot account for the other two forms in this way. Attempts to find systematic structural absences among the observed reflections of the other two forms, which would give clues about twinning, have so far been unsuccessful. Work on the bornite puzzle is being continued.

SILICATES

Pyroxenes

N. Morimoto et al.

The determination of accurate crystal structures of rock-forming minerals of known chemical composition formed un-

der various conditions has become an important field in mineralogy. Crystallographers have carried out excellent studies on feldspars and amphiboles. The corresponding study of pyroxene structure in relation to chemical composition and genesis, however, has not yet been made in spite of recent developments in the petrological study of minerals of this group. Some accurate measurements of the cell dimensions have been made by Hess and Kuno. To cover the gap between the petrology and crystallography of pyroxenes the X-ray study of pyroxenes of various compositions has been undertaken.

The pyroxenes present exceptional complexity both of crystalline modifications and of chemical composition. The most important natural pyroxenes, however, occur in the ternary system $\text{MgSiO}_3\text{--FeSiO}_3\text{--CaSiO}_3$, with less than 50 mole per cent CaSiO_3 , although a minor amount of replacement of other cations (Al, Ti, etc.) usually takes place. Over most of this field, the pyroxenes are monoclinic and an orthorhombic modification is more stable only when there is less than 10 per cent CaSiO_3 at low temperature. In the $\text{MgSiO}_3\text{--CaSiO}_3$ join, however, Atlas (1952) and Schairer and Boyd (1957) showed that protoenstatite, an orthorhombic modification other than the usual enstatite, is stable at high temperature, and clinoenstatite is a metastable form.

Clinopyroxenes. Clinopyroxene in the ternary system $\text{MgSiO}_3\text{--FeSiO}_3\text{--CaSiO}_3$ can be divided into two different types on the basis of space group, (a) diopside-hedenbergite join (augite type), (b) clinoenstatite-clinoferrosilite join (pigeonite type).

The augite-type clinopyroxenes belong to space group $C_{2h}^6\text{--}C_{21}/c$ (Warren and Bischoe, 1931). Their β angle and cell volume change continuously as the replacement of metallic atoms takes place, suggesting the existence of complete solid solution in this type. In the pigeonite-type clinopyroxenes, however, the β angle is nearly constant throughout the type, though the cell volume changes gradually. The space group

of this type was found to be $C_{2h}^5-P2_1/a$ (Morimoto, 1956; Gay and Bown, 1957).

The existence of a miscibility gap between diopside and clinoenstatite (Atlas, 1952; Schairer and Boyd, 1957) was confirmed. It is not certain, however, at what compositions the solvus intersects the solidus in clinopyroxenes, though it is believed that a complete solid solution between the augite- and pigeonite-type clinopyroxene begins to exist when the pyroxenes become rich in Fe^{+2} . The following information (obtained jointly with T. Ito, Tokyo University) throws some light on this problem.

Phenocrysts in an andesite from Hakone, Japan, studied by the Weissenberg method, show very minute intergrowths of pigeonite ($Wo_{16}En_{45}Fs_{39}$) and augite ($Wo_{32}En_{37}Fs_{31}$) having the (001) plane in common (Morimoto, 1956). Some specimens from the same locality show an unusual twinlike structure of two such intergrowths of pigeonite and augite as in figure 47. The b axes of the four crystals in this edifice are the same not only in orientation but also in length. The c axes of the two pigeonite crystals enclose an angle of 1° , and of the two augite crystals, an angle of 3.6° . This combination of two pigeonite-augite intergrowths can be interpreted to be in "twin" relation on a plane whose indices are not integers but which is close to (100) for all four crystals. Although, of course, we cannot refer to this edifice as a twin, we call it a "pseudo-twin." On the microscope, the sample used in X-ray studies does simulate polysynthetic twinning of pigeonite parallel to the (100) plane, with rather broad lamellae. Between pigeonite bands there are narrow strips, so narrow in fact that it is impossible optically to decide whether they are pigeonite or augite. To judge from X-ray experiments, they are probably augite.

The existence of this polysynthetic twinlike structure of pigeonite and augite can reasonably be understood as an exsolution product of a mixed crystal. Clinopyroxene,

originally crystallized as a homogeneous phase, later unmixed to pigeonite and augite. The composition plane of the pigeonites and augites is supposed to be the (100) plane of the homogeneous phase. The coincidence of the b axes of pigeonite and augite also suggests that they have unmixed from one high-temperature pyroxene.

It is possible to say from the above results that there is a mixed crystal between augite and pigeonite at a high temperature but that unmixing to augite and pi-

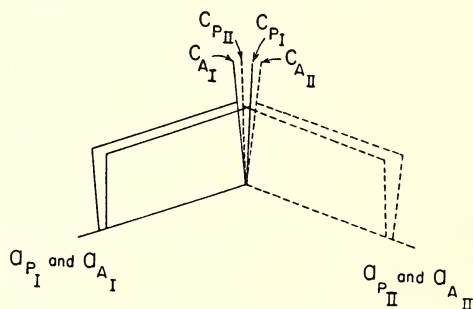


Fig. 47. Unusual pseudo-twin of pigeonite and augite, which suggests exsolution from homogeneous clinopyroxene. a_{P_I}, c_{P_I} : the a and c axes of pigeonite I. $a_{P_{II}}, c_{P_{II}}$: the a and c axes of pigeonite II. a_{A_I}, c_{A_I} : the a and c axes of augite I. $a_{A_{II}}, c_{A_{II}}$: the a and c axes of augite II. Pigeonite I and augite I have the (001) plane in common. The relation between pigeonite II and augite II is the same as that of pigeonite I and augite I. The composite plane is supposed to be the (100) plane of the original clinopyroxene.

geonite takes place on cooling at the composition of $Wo_{18}En_{44}Fs_{38}$. (This composition is estimated from the ratio of the intensities of the reflections of pigeonite and augite on X-ray photographs.) We are planning heating and quenching experiments on phenocrysts that consist of pseudo-twins. The case of pseudo-twinning reported here is the second one on record. The first one was found in a feldspar specimen (Spencer N) by G. Donnay and J. D. H. Donnay (Year Book 53).

Clinopyroxene of the pigeonite type has a primitive lattice and shows the reflections hkl with $(h+k)$ odd, which do not occur in the augite-type clinopyroxenes. In fig-

ure 48 parts of the powder patterns of four materials belonging to this type are shown, together with their chemical compositions. The intensity of the reflection (231) decreases as the Ca and Fe contents increase, and almost disappears in the pat-

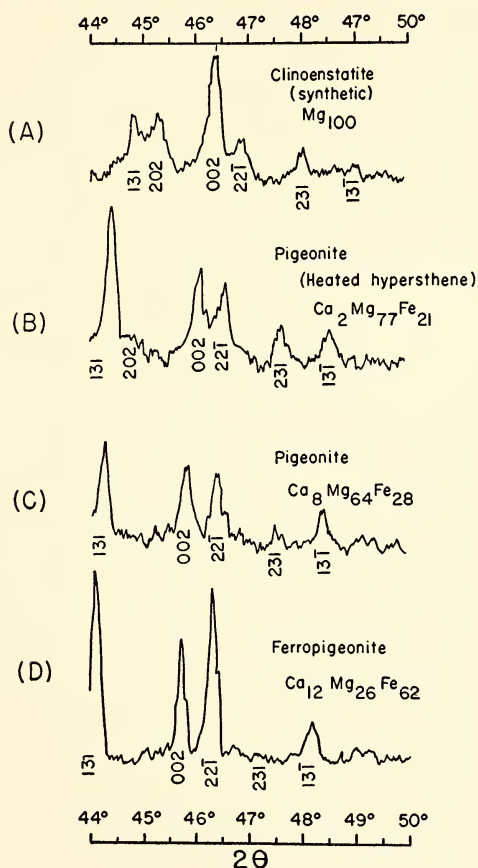


Fig. 48. The diffraction patterns of (A) synthetic clinoenstatite; (B) pigeonite transformed from hypersthene, Kamakura, Japan (heated at 1400° C for 16 hours in a vacuum silica tube); (C) pigeonite, Usugoyazawa, Japan; (D) ferropigeonite, Ashio, Japan, taken by Norelco X-ray diffractometer using Fe-radiation.

tern of ferropigeonite of the composition $Wo_{12}En_{26}Fs_{62}$, suggesting a *C*-centered lattice, which is that of the augite-type clinopyroxenes.

The single-crystal photographs of this ferropigeonite, however, give a fairly strong reflection (231) and other, additional reflections with $(h+k)$ odd, which

are proof of the primitive lattice. These additional reflections are usually somewhat diffuse (Gay, 1957) and seem to have streaks along the *a* axis. On the other hand, pigeonite of composition $Wo_{16}En_{45}Fs_{39}$ shows very sharp additional reflections. The intensity of the additional reflections is generally weaker than that of the usual reflections ($h+k=2n$) in the ferropigeonite, and even weaker in the pigeonite. This observation suggests that the intensity of the additional reflections does not depend on the Fe content.

Thus we can conclude that (1) the intensity of the additional reflections depends on the amount of Ca in the structure, and (2) the diffuseness of the additional reflections depends on the amount of Fe.¹¹

The accurate structure analysis of clinoenstatite and ferropigeonite is now in progress (with H. T. Evans and D. E. Appleman, U. S. Geological Survey) and should clarify the following questions: (1) To what extent are the atomic coordinates of clinoenstatite and ferropigeonite different from those of diopside? (2) To what extent are the atomic coordinates of clinoenstatite different from those of ferropigeonite? (3) What is the configuration of cations such as Ca, Mg, and Fe atoms in ferropigeonite structure? (4) Why does the gradual change of intensity and diffuseness of the additional reflections in the pigeonite-type clinopyroxenes take place?

Specimens of clinoenstatite were obtained by heating Webster 26 bronzite ($En_{91}Fs_9$) and Bishopville enstatite at 1400° C for 24 hours. They usually show fine polysynthetic twinning on the (100) plane, suggesting the transition from protoenstatite, which is supposed to be a stable high-temperature form. The single

¹¹ According to Drs. Gay and Bown, Cambridge, England (private communication), the diffuseness of these reflections does not change after 2 days of heating at 1000° C and quenching in air. Therefore, they conclude, the diffuseness does not depend on the rate of cooling of the minerals.

crystals of ferropigeonite ($\text{Wo}_{12}\text{En}_{26}\text{Fs}_{82}$) were obtained from a dike at Ashio, Japan, by Kuno.

The structures of clinoenstatite and ferropigeonite were obtained by deforming the structure of diopside, giving the additional reflections. Their structures are now in the stage of refinement using ($hk0$), ($h0l$), and ($0kl$) reflections.

Orthopyroxenes. The structure analysis of ferropigeonite suggests that Mg, Fe, and Ca atoms are randomly distributed between two crystallographically different positions. In orthopyroxenes, which are a stable form at low temperature, we can expect some ordering of cations into different positions. Furthermore, T. Ito (1950), on the basis of his twinning theory, proposed a structure for bronzite ($\text{En}_{84}\text{Fs}_{16}$) slightly different from that proposed by Warren and Modell for hypersthene ($\text{En}_{70}\text{Fs}_{30}$). Refinement of enstatite (Bishopville, En_{100}) and ferrohypersthene (Hirogawara, $\text{En}_{55}\text{Fs}_{45}$) structures is now under way.

In the course of the study of orthopyroxenes, it was found that orthopyroxenes of plutonic origin (Great Dike, Webster, etc.) always show some additional reflections, such as (012), (032), etc., though they are very weak. These reflections are prohibited by the extinction rule of the space group of orthopyroxenes $D_{2h}^{15}-Pbca$. In orthopyroxenes of volcanic or meteoritic origin (Bishopville, Bonin Island, Hirogawara, etc.), however, it was impossible to observe these reflections even in photographs of long exposure. These reflections, therefore, can be interpreted as (112), ($\bar{1}32$), etc., of the diopsidic pyroxene on the basis of the exsolution of diopsidic pyroxene from hypersthene by cooling and the intergrowth of the diopsidic pyroxene and hypersthene on the (100) plane.

Structural relation between enstatite, clinoenstatite, and protoenstatite. It is important to study the mechanism of transition from orthopyroxene to clinopyroxene. Diffuse reflections of monoclinic symmetry along the a^* axis can be observed even in

natural enstatite; the symmetry of the diffuse reflections changes to orthorhombic when the mineral inverts to clinoenstatite by heating. The study of the transition mechanism between clinoenstatite, enstatite, and protoenstatite and the interpretation of the diffuse reflections can be undertaken by means of heating experiments on single crystals, once the precise structure of these minerals is known. Some heating experiments have been conducted this year, and the work will be continued during the coming year.

Synthetic Mica of Type 3M

G. Donnay and P. Kingman

Although numerous studies have been made on the mica group of minerals because of their abundance and geological importance, precise crystal-structure data are still unavailable. Information about bond angles and interatomic distances is still of a comparatively crude nature.

A structure determination of a synthetic mica would be of considerable value, since one of the chief features of the group is the multiplicity of isomorphous substitutions, and information about bond angles, lengths, etc., obtained from a single-crystal study would be of great assistance in understanding the crystal chemistry of these substitutions.

In the extensive study of the mica system, $\text{KFe}_3(\text{FeSi}_3\text{O}_{10})(\text{OH})_2$ has been synthesized (Wones and Eugster) in sheets thick enough to be used for X-ray studies by single-crystal methods. This synthetic mica has distinct advantages for a structure determination. First, the composition of the synthetic mineral is much more accurately known than that of naturally occurring micas. Second, the substitution of *nothing but* Fe^{+++} atoms for some of the Si atoms in tetrahedral coordination—a substitution apparently not occurring in nature—offers the possibility of determining whether such substitutions are ordered or disordered. In natural micas the element that substitutes for silicon is aluminum,

whose atomic weight is so similar to that of silicon that distinguishing between the two by X-ray diffraction is difficult. It is also possible that the substance may have interesting magnetic properties due to the presence of Fe^{++} and Fe^{+++} , and examination by neutron diffraction might prove fruitful.

Crystals have been examined on precession and Weissenberg cameras with $\text{Fe K}\alpha$ ($\lambda=1.9373 \text{ \AA}$) and $\text{Mo K}\alpha$ ($\lambda=0.7108 \text{ \AA}$) radiations. The lattice is metrically hex-

agonal, but the symmetry is *monoclinic*, with diffraction aspect C^* . The cell dimensions are: $a=5.43_4$, $b=9.40_4$, $c=30.4_9 \text{ \AA}$, all ± 0.3 per cent, $\beta=90^\circ 0'$. For an orthohexagonal lattice $b=2a \cos 30^\circ=9.41_2 \text{ \AA}$, which is equal within experimental error to the measured b value. The hexagonal plates, with excellent cleavage (001), as well as the cell dimensions, indicate a mica-type structure with a cell three layers high.

MISCELLANEOUS ADMINISTRATION

Petrologists' Club

The Petrologists' Club held an increased number of meetings this year and revived its annual field trip. Ores and ore solutions, together with isotope studies, were subjects discussed at the majority of the meetings. A field trip to examine the metamorphic rocks in the Fairfax quadrangle, Virginia, was led by Charles Milton and Edward C. T. Chao. D. B. Stewart and E. H. Roseboom, Jr., were elected to serve as chairmen for 1959. Speakers whom the club was privileged to hear this past season are listed below.

" $\text{O}^{16}/\text{O}^{18}$ ratios in coexisting minerals from various geologic deposits," by Sam Epstein (California Institute of Technology).

"Some observations on natural and synthetic carbonates," by Julian R. Goldsmith (University of Chicago).

"Composition of magmatic gases at high temperatures," by Konrad B. Krauskopf (Stanford University).

"Thermochemical data and its application to limestone replacement," by H. Holland (Princeton University).

"Fractionation of deuterium in earth processes," by Irving Friedman (U. S. Geological Survey).

"Some aspects of current sulfide research," by G. Kullerud, E. H. Roseboom, Jr., and R. G. Arnold (Geophysical Laboratory).

"Fluid pressure hypothesis and overthrusting in western Wyoming," by W. W. Rubey (U. S. Geological Survey).

"Sediments of California marine basins," by Kenneth O. Emery (University of Southern California).

Seminars

The Laboratory continued its weekly series of seminars, with papers presented largely by staff members and concerned mainly with discussions of work in progress. The following talks were given by guest speakers from outside the Laboratory:

"The olivine-spinel transition and its bearing on the composition of the mantle," by A. E. Ringwood (University of Melbourne, Australia).

"Standard free energies of formation deduced from mineral occurrences," by R. M. Garrels (Harvard University).

"Infra-red spectroscopy of inorganic systems," by E. R. Lippencott (University of Maryland).

"Activation analysis applied to geochemical problems," by George W. Reed (Argonne National Laboratory).

"Some variations in $\text{O}^{18}/\text{O}^{16}$ ratios in natural minerals," by Sam Epstein (California Institute of Technology).

"Attenuation of small-amplitude stress waves in solids," by Gordon J. F. MacDonald (Massachusetts Institute of Technology).

"Problems in mineral facies," by James B. Thompson (Harvard University).

"Carbon-14 dating and the Pleistocene geology of Europe," by Hessel de Vries (University of Groningen, Netherlands).

"Geochemical prospecting," by H. E. Hawkes (University of California, Berkeley).

"Geochemistry of the isotopes of nitrogen," by T. Hoering (University of Arkansas).

"Geochemical indications of the environment of deposition of sedimentary rocks," by M. L. Keith (Pennsylvania State University).

"Magnetic structure of chalcopyrite as determined by neutron diffraction," by L. M. Corliss (Brookhaven National Laboratory) and G. Donnay (Geophysical Laboratory).

"Some aspects of the geochemistry of sulfur isotopes," by Wayne U. Ault (U. S. Geological Survey).

"Research in ceramics," by A. T. Green (British Ceramic Research Association).

"The dating of marine sediments: Problems and progress," by Gustaf O. Arrhenius (Scripps Institution of Oceanography).

"Some aspects of the geochemistry of petroleum," by W. E. Hanson (Mellon Institute).

"The dynamic consequences of phase transitions in the mantle," by Gordon J. F. MacDonald (Massachusetts Institute of Technology).

"Operation Rainier," by G. W. Morey (U. S. Geological Survey).

"The application of optical methods to the study of imperfect structures," by H. Lipson (College of Science and Technology, Manchester).

Johns Hopkins University and Geophysical Laboratory Graduate Seminars on "Researches in Geochemistry"

During the academic year 1957-1958 the Geophysical Laboratory arranged a weekly series of graduate seminars on research in geochemistry at the Johns Hopkins University. Many of the speakers also visited and gave talks at the Geophysical Laboratory. The content of these lectures has been assembled for publication by John Wiley & Sons in a book entitled *Researches in Geochemistry*.

Speakers and topics included: Gunnar Kullerud (Geophysical Laboratory), "Sulfide systems as geological thermometers"; Hans P. Eugster (Geophysical Laboratory), "Reduction and oxidation in metamorphism"; Robert M. Garrels (Harvard University), "Rates of geochemical reactions at low temperatures and pressures"; George R. Tilton (Geophysical Laboratory), "Geochronology"; George W. Reed (Argonne National Laboratory), "Activation analysis applied to geochemical prob-

lems"; Sam Epstein (California Institute of Technology), "Variation of the O^{18}/O^{16} ratio in nature"; Julian R. Goldsmith (University of Chicago), "Some aspects of the geochemistry of carbonates"; Willard F. Libby (Atomic Energy Commission and Geophysical Laboratory), "Tritium in hydrology and meteorology"; Konrad B. Krauskopf (Stanford University), "The use of equilibrium calculations in finding the composition of a magmatic gas phase"; Gordon J. F. MacDonald (Massachusetts Institute of Technology), "Chondrites and the chemical composition of the earth"; James B. Thompson (Harvard University), "The phase rule and metasomatism"; Francis R. Boyd (Geophysical Laboratory), "Hydrothermal investigations of amphiboles"; Paul B. Barton, Jr. (U. S. Geological Survey), "The chemical environment of ore deposition and the problem of low-temperature ore transport"; H. E. Hawkes (University of California), "Geochemical prospecting"; Wayne U. Ault (U. S. Geological Survey), "Sulfur isotopic fractionation in geochemical processes"; M. L. Keith (Pennsylvania State University), "Geochemical indicators of marine and fresh-water sediments"; Philip H. Abelson (Geophysical Laboratory), "Geochemistry of organic substances"; Charles Milton (U. S. Geological Survey), "Mineral assemblages of the Green River formation"; Felix Chayes (Geophysical Laboratory), "Diffraction effects of short-range ordering in layered sequences"; William E. Hanson (Mellon Institute of Industrial Research), "Some aspects of the geochemistry of petroleum"; Gustaf O. Arrhenius (Scripps Institution of Oceanography), "Sedimentary processes and records in the ocean"; Sydney P. Clark, Jr. (Geophysical Laboratory), "Equations of state and polymorphism at high pressure"; Kenneth O. Emery (University of Southern California), "Sediments of California marine basins"; Hessel de Vries (University of Groningen), "Measurement and use of natural radiocarbon."

Symposium on C¹⁴ Dating, Pleistocene Stratigraphy, and Archaeologic Chronology

A one-day conference devoted mainly to problems in Pleistocene geology was held at the Geophysical Laboratory on May 22, 1958. Professor Hessel de Vries (University of Groningen, Netherlands) discussed the results of recent C¹⁴ work at Groningen with particular reference to Pleistocene geology in western Europe. Professor Friedrich Brandtner (currently a Fellow at Yale University) gave an account of his field observations in the Pleistocene of western Europe, and compared them with the C¹⁴ results.

After a buffet luncheon, Dr. Ralph Solecki (Smithsonian Institution) spoke on C¹⁴ dates from the Shanidar cave in Iraq and their significance to Pleistocene geology. Professor R. F. Flint (Yale University) discussed Pleistocene problems in North America, especially the studies that have been made at Searles Lake. The glacial sequence of North America was discussed by Dr. Meyer Rubin (U. S. Geological Survey). Dr. W. S. Broecker (Lamont Geological Observatory) presented C¹⁴ dates from pluvial lakes in the Great Basin of western North America. Professor de Vries then reviewed data on the fluctuation of C¹⁴ concentration in samples of modern carbon. Finally, Dr. Irving Friedman (U. S. Geological Survey) spoke on the hydration of obsidian artifacts as a new dating tool.

Besides personnel from the Geophysical Laboratory, the conference was attended by approximately twenty-five representatives from the U. S. Geological Survey, the Smithsonian Institution, the Lamont Geological Observatory, Yale University, and the Peabody Foundation.

Lectures

During the report year staff members were invited to present lectures as follows:

P. H. Abelson lectured at a symposium on organic geochemistry sponsored by the

American Petroleum Institute at Dallas, Texas; the D. C. Chapter of the American Institute of Chemists; the Department of Genetics, Cold Spring Harbor, N. Y.; the National Bureau of Standards; a symposium on biochemical origins at the San Francisco meeting of the American Chemical Society; the College of Mineral Industries at Pennsylvania State University; the Kansas Chapter of Phi Sigma and the Department of Bacteriology at the University of Kansas; and at the Army Conference on Basic and Applied Research and Component Development at Operations Research Office of Johns Hopkins University.

F. R. Boyd addressed the Washington Academy of Sciences and Howard University, D. C.

F. Chayes lectured at the Department of Geology and Geophysics, Massachusetts Institute of Technology.

H. P. Eugster again served as Lecturer in the Department of Geology, Johns Hopkins University, each Friday during the academic year 1957-1958. He also addressed the Department of Geology and the Institute of Geophysics at the University of California at Los Angeles.

G. Kullerud lectured at the New York meeting of the American Institute of Mining and Engineering.

N. Morimoto presented a symposium on the crystal structure of borax to the Washington Crystal Colloquium.

J. F. Schairer addressed a meeting of the Philosophical Society of Washington.

G. R. Tilton gave several lectures on the geochemistry of stable isotopes at Johns Hopkins University.

H. S. Yoder lectured at the Conference on the Structure and Properties of Natural and Synthetic Minerals, Pennsylvania State University; the Sixth Clay Conference at the University of California, Berkeley; the Department of Geology, Michigan State University; and the Geology Club, McGill University, Montreal. He also gave a series of six lectures at the Summer Institute in Geology for college teachers

at the University of Illinois. Three lectures were given at the Department of Geology, University of Minnesota, Minneapolis.

The "Summary of Published Work" below briefly describes the papers published in scientific journals during the report year. In addition, the following papers are now prepared for publication: G. Donnay, "Crystal data on chlorophyll *a*"; J. D. H. Donnay and G. Donnay, "Sine table for indexing powder patterns"; G. Donnay and J. G. Smith, "Calibration sights for X-ray powder camera"; G. Kul-

lerud and G. Donnay, "Natural and synthetic ferroselite: A roentgenographic mimesis of rammelsbergite"; J. F. Schairer, "Phase equilibria in silicate systems"; J. R. Smith, "The optical properties of heated plagioclases"; G. R. Tilton, "Isotopic composition of lead from tektites"; G. R. Tilton, G. W. Wetherill, G. L. Davis, and C. A. Hopson, "Ages of minerals from the Baltimore gneiss"; O. F. Tuttle and N. L. Bowen, "The origin of granites in the light of experimental studies in the system $\text{NaAlSi}_3\text{O}_8\text{-KAlSi}_3\text{O}_8\text{-SiO}_2\text{-H}_2\text{O}$ "; H. S. Yoder, "Experimental studies on micas: A synthesis."

SUMMARY OF PUBLISHED WORK

- (1269) The system water-nepheline-albite: A theoretical discussion. G. W. Morey. *Am. J. Sci.*, 255, 461-480 (1957).

At an invariant or quintuple point in a three-component system, five pressure-temperature curves of univariant equilibria intersect. The sequence of these *P-T* curves, that is, the order in which they are met in either their stable portions or their metastable prolongations, is determined by the compositions of the phases at that point. This proposition is proved, and its application is illustrated by a discussion of possible *P-T* curves and invariant points in the system water-nepheline-albite.

- (1270) Isotopic ages of zircon from granites and pegmatites. G. R. Tilton, G. L. Davis, G. W. Wetherill, and L. T. Aldrich. *Trans. Am. Geophys. Union*, 38, 360-371 (1957).

Isotopic lead age determinations have been made on 13 zircons obtained from rocks 185 to 1400 million years old. Concordant or nearly concordant ages are found for all the samples containing no detectable common lead, and discordant ages are found for most, if not all, of the samples containing common lead. A comparison is made between the concordant isotopic age patterns given by 3 zircons from the Grenville subprovince in Ontario and the discordant patterns given by 3 zircons from the Cordilleran region of western United States. This comparison indicates that the discordant ages can be related to the

recent orogenies that occurred in the Cordilleran region. The Grenville is a stable shield area. There is no relation between the agreement of the isotopic ages of zircon and crystal size, amount of radiation damage, or optical appearance—that is, zoning, cloudiness, or inclusions.

When a discordant age result is compared with the potassium-argon and rubidium-strontium ages of associated mica the $\text{Pb}^{207}\text{-Pb}^{206}$ age is found to be the closest to the mica age. Isotopic ages are compared with the simpler α -lead and chemical-lead ages, which do not require isotopic analysis of lead. The nonisotopic ages are approximately correct for zircons that have concordant isotopic ages but are in error when discordant isotopic ages are found. No explanation is offered as to why the mica ages are apparently unaffected by the process or processes that altered the zircon ages. An understanding of this phenomenon would doubtless provide valuable information about the post-crystallization history of the samples.

- (1271) Melting relations of the common rock-forming oxides. J. F. Schairer. *J. Am. Ceram. Soc.*, 40, 215-235 (1957).

Geological science is concerned with the nature of the minerals of the earth and particularly with the processes by which earth materials have been changed and modified. Laboratory studies of the melting behavior of the common rock-forming oxides have been an important adjunct to the observations of

the field geologist. For the past fifty years investigators at the Geophysical Laboratory have been obtaining quantitative information on the melting relations of many of the important rock-forming minerals. These studies of the fundamental chemistry of igneous and metamorphic rocks have yielded much information of value to ceramists, metallurgists, and mineral technologists. This paper summarizes the most important phase-equilibrium studies of unary, binary, ternary, quaternary, and portions of quinary systems of the common rock-forming oxides— SiO_2 , Al_2O_3 , FeO , Fe_2O_3 , CaO , MgO , Na_2O , and K_2O .

- (1272) The crystalline modifications of $\text{NaAlSi}_3\text{O}_8$. W. S. MacKenzie. *Am. J. Sci.*, 255, 481–516 (1957).

X-ray studies of synthetic albites reveal a wide variation in lattice parameters of the crystals, depending on the conditions of crystallization.

A glass of composition $\text{NaAlSi}_3\text{O}_8$ has been crystallized in the presence of water vapor under pressure, at temperatures between 450° and 1000°C for varying periods of time. X-ray study of the crystals reveals that at each temperature the crystals formed after a few hours are similar to high-temperature albite in lattice parameters. Experiments of longer duration, however, show that the lattice parameters of the initially formed crystals change gradually, finally reaching a steady value characteristic of the temperature of crystallization. The results suggest that for each temperature there is a stable crystalline form of $\text{NaAlSi}_3\text{O}_8$ intermediate between high-temperature albite and low-temperature albite, high-temperature albite being stable only above about 1000°C and low-temperature albite only below about 450°C .

Previous investigations have indicated that some natural potassium feldspars may have crystallized metastably as the high-temperature monoclinic modification sanidine, and subsequently inverted to the low-temperature triclinic modification microcline. The experiments reported here support a similar conclusion in respect to sodium feldspar, namely that some natural albites now in the low-temperature form may have crystallized metastably in the high-temperature form and gradually inverted to the low-temperature form. The variations in the properties of albites

from low-temperature veins of Alpine type are consistent with this interpretation.

- (1273) Olivine X-ray determinative curve. H. S. Yoder, Jr., and Th. G. Sahama. *Am. Mineralogist*, 42, 475–491 (1957).

The (130) spacing of 31 chemically analyzed natural olivines and 7 synthetic olivines has been measured. A determinative curve has been calculated from 26 of the chemically analyzed natural olivines:

$$\text{Fo (mole per cent)} = 4233.91 - 1494.59d_{130}.$$

The fictive end points are d_{130} (Fo = 100) = 2.7659 and d_{130} (Fo = 0) = 2.8328. The error attached to an individual measurement ranges from 3 to 4 mole per cent, depending on the composition.

Portions of the powder X-ray diffraction patterns for synthetic forsterite and synthetic fayalite have been indexed. The cell constants, density, and molar volumes are given.

- (1274) Some aspects of paleobiochemistry. P. H. Abelson. *Annals N. Y. Acad. Sci.*, 69, 276–285 (1957).

Organic chemicals of biological origin are being found in an increasing number of fossils, sediments, and sedimentary rocks. The existence of these paleobiochemicals has special significance in many areas of science. It raises real possibilities of achieving better knowledge of the comparative biochemistry of creatures long extinct, including some of the earliest forms of life. The occurrence of these ancient substances permits study of organic chemicals under circumstances in which the rate of reaction is known to be less than 1 part in 10^{17} per second. Knowledge of the stability of these compounds is important to the question of the origin of life, since most speculations assume the accumulation of a pool of organic substance during a long pre-life stage.

- (1275) Experimental error in determining certain peak locations and distances between peaks in X-ray (powder) diffractometer patterns. F. Chayes and W. S. MacKenzie. *Am. Mineralogist*, 42, 534–547 (1957).

The random errors inherent in two common types of X-ray diffractometer measurement have been estimated. Most of the error is apparently associated with operations con-

tingent upon setting up the specimen and getting the instrument in motion. Although significant mean differences were detected between mounts of different type, different mounts of the same type gave agreement well within experimental error. Results obtained at scale factor 4 were indistinguishable from those obtained at scale factor 2. Scanning direction exerts a small but significant effect on estimates of distances between peaks. The exact numerical results apply strictly only to the specific operator-instrument combination that generated the data. The evaluation procedures should be widely applicable.

- (1276) Relations between composition of ore minerals and ore solutions. H. L. Barnes and G. Kullerud. *Econ. Geol.*, 52, 825-830 (1957).

At least four independent variables (pressure, temperature, ionic species, and activity coefficients) must be quantitatively evaluated in order to deduce the composition of an ore solution from the composition of precipitated minerals. Application of the derived functions is limited to the cases in which equilibrium during precipitation and absence of post-depositional changes can be demonstrated. These conditions so greatly limit the usefulness of natural data that additional relations between aqueous solutions and their precipitates are needed before unique answers to the variables can be calculated for individual deposits. At present, sufficient experimental data for derivation of the required functions are lacking.

- (1277) Annual report of the Director for 1956-1957.
- (1278) Disorder in a crystalline condensed phosphate. J. W. Gryder, G. Donnay, and H. M. Ondik. *Acta Cryst.*, 11, 38-40 (1958).

$\text{Na}_2\text{P}_4\text{O}_{11}$ crystallizes in two distinct forms. Form I is monoclinic with $a=30.7$, $b=6.77$, $c=7.12$ Å, $\beta=94^\circ 6'$; space group $P2_1/a$. Form II is also monoclinic but markedly pseudo-orthorhombic with $a=18.74$, $b=14.79$, $c=7.03$ Å, $\beta=90^\circ 0'$; space group $B2/a$ or Ba . Reflections from form II with l odd consist of diffuse circular disks oriented normal to the c^* axis. In the structure postulated for form II, rings of four phosphate tetrahedra

are linked into chains by the sharing of an oxygen between adjacent rings.

- (1279) The transition between the low- and the high-temperature form of sodium tripolyphosphate. G. W. Morey. *J. Am. Chem. Soc.*, 80, 775 (1958).

Sodium tripolyphosphate was heated in closed tubes with 0.1 per cent $\text{Na}_5\text{P}_3\text{O}_{10} \cdot 6\text{H}_2\text{O}$, corresponding to 0.023 per cent H_2O , for periods of about a month. Under these conditions at 409°C and below, $\text{Na}_5\text{P}_3\text{O}_{10}\text{I}$ changed to $\text{Na}_5\text{P}_3\text{O}_{10}\text{II}$, while $\text{Na}_5\text{P}_3\text{O}_{10}\text{II}$ remained unchanged; and at 425°C , $\text{Na}_5\text{P}_3\text{O}_{10}\text{II}$ changed to $\text{Na}_5\text{P}_3\text{O}_{10}\text{I}$, while $\text{Na}_5\text{P}_3\text{O}_{10}\text{I}$ remained unchanged. The two forms are enantiotropic with a transition temperature of $417^\circ \pm 8^\circ\text{C}$.

- (1280) Crystal and twin structure of digenite, Cu_9S_5 . G. Donnay, J. D. H. Donnay, and G. Kullerud. *Am. Mineralogist*, 43, 228-242 (1958).

Digenite undergoes a rapidly reversible and nonquenchable transformation between 60° and 65°C . Digenite was synthesized in octahedra modified by small cube faces. Single-crystal methods yield a cubic cell whose edge a is equal to five times the literature value: $a=27.71$ Å ± 0.3 per cent. With 100 Cu_9S_5 per cell, the calculated specific gravity is 5.715, against 5.6 observed. Only HKL reflections observed are of the type $10m \pm L$, $10n \pm L$, L , with m and n integers. The numerous structural absences are explained by twinning; the octahedron consists of four rhombohedral crystals, oriented with their hexagonal c axes along the body diagonals and their hexagonal a axes along the face diagonals of the simulated cubic cell. The twin axis is $[337]$ in rhombohedral notation; twin index 5; twin obliquity 0. The rhombohedral cell ($a=16.16$ Å, $\alpha=13^\circ 56'$) contains one Cu_9S_5 . The diffraction aspect is R^{**} . The pronounced pseudo-cube, $a'=a/5=5.54$ Å, is explained as follows. Only those reflections whose cubic indices HKL are multiples of 5 receive contributions from all four crystals of the twin; they are the only reflections, moreover, to which sulfur atoms contribute. Space group $R\bar{3}m$ leads to a tentative structure in which all atoms lie on the threefold axis of the rhombohedral cell, in positions xxx . For the five sulfur atoms, the values of x are: 0, $\pm 1/5$, $\pm 2/5$; for the nine copper atoms: $1/2$, ± 0.060 , ± 0.133 , ± 0.250 , ± 0.350 .

(1281) A possible explanation of the δ_c separations in intermediate plagioclase. F. Chayes. *Acta Cryst.*, 11, 323–324 (1958).

The δ_c “separation” in intermediate plagioclase varies with composition as the reciprocal of the average run length of the major constituent in a two-element random run sequence containing $Na\ A$'s and $N\beta\ B$'s, where a is the proportion of tetrahedral sites occupied by Al. The case corresponds to virtually complete short-range disorder in at least one direction.

(1283) Heterogeneous reactions involving oxidation and reduction at high pressures and temperatures. H. P. Eugster. *J. Chem. Phys.*, 26, 1760–1761 (1957).

This brief communication describes an experimental procedure developed for investigation of stabilities and phase relations of hydrous iron silicates at high water pressures. The chemical potential of oxygen of the charge is controlled with the aid of a number of calibrated oxygen buffers.

BIBLIOGRAPHY

Abelson, P. H. Some aspects of paleobiochemistry. *Annals N. Y. Acad. Sci.*, 69, 276–285 (1957).

Aldrich, L. T. See Tilton, G. R.

Barnes, H. L., and G. Kullerud. Relations between composition of ore minerals and ore solutions. *Econ. Geol.*, 52, 825–830 (1957).

Chayes, F. A possible explanation of the δ_c separations in intermediate plagioclase. *Acta Cryst.*, 11, 323–324 (1958).

Chayes, F., and W. S. MacKenzie. Experimental error in determining certain peak locations and distances between peaks in X-ray (powder) diffractometer patterns. *Am. Mineralogist*, 42, 534–547 (1957).

Davis, G. L. See Tilton, G. R.

Donnay, G., J. D. H. Donnay, and G. Kullerud. Crystal and twin structure of digenite, Cu_9S_5 . *Am. Mineralogist*, 43, 228–242 (1958).

Donnay, G. See also Gryder, J. W.

Donnay, J. D. H. See Donnay, G.

Eugster, H. P. Heterogeneous reactions involving oxidation and reduction at high pressures and temperatures. *J. Chem. Phys.*, 26, 1760–1761 (1957).

Gryder, J. W., G. Donnay, and H. M. Ondik.

Disorder in a crystalline condensed phosphate. *Acta Cryst.*, 11, 38–40 (1958).

Kullerud, G. See Barnes, H. L.; Donnay, G.

MacKenzie, W. S. The crystalline modifications of $NaAlSi_3O_8$. *Am. J. Sci.*, 255, 481–516 (1957).

MacKenzie, W. S. See also Chayes, F.

Morey, G. W. The system water–nepheline–albite: A theoretical discussion. *Am. J. Sci.*, 255, 461–480 (1957).

Morey, G. W. The transition between the low- and the high-temperature form of sodium tripolyphosphate. *J. Am. Chem. Soc.*, 80, 775 (1958).

Ondik, H. M. See Gryder, J. W.

Sahama, Th. G. See Yoder, H. S.

Schairer, J. F. Melting relations of the common rock-forming oxides. *J. Am. Ceram. Soc.*, 40, 215–235 (1957).

Tilton, G. R., G. L. Davis, G. W. Wetherill, and L. T. Aldrich. Isotopic ages of zircon from granites and pegmatites. *Trans. Am. Geophys. Union*, 38, 360–371 (1957).

Wetherill, G. W. See Tilton, G. R.

Yoder, H. S., Jr., and Th. G. Sahama. Olivine X-ray determinative curve. *Am. Mineralogist*, 42, 475–491 (1957).

PERSONNEL

Scientific Staff

Director: P. H. Abelson.

Retired Associates: H. E. Merwin, *Petrologist*; E. G. Zies, *Chemist*.

Staff Associate: G. J. F. MacDonald.

Physical Chemists: F. R. Boyd, J. F. Schairer, G. R. Tilton.

Petrologists: F. Chayes, J. W. Greig, W. S. MacKenzie,¹ H. S. Yoder, Jr.

Geochemists: G. L. Davis, H. P. Eugster, G. Kullerud.

Geophysicist: S. P. Clark, Jr.

Physicist: J. L. England.

Crystallographer: G. Donnay.

Guest Investigators: P. B. Barton, Jr., U. S. Geological Survey; Hessel de Vries, University of Groningen; H. Faul, U. S. Geological Survey; H. R. Gault, Lehigh University; J. G. Palacas, University of Minnesota; C. R. Robbins, National Bureau of Standards; C. Spangler, U. S. Geological Survey; D. B. Stewart, U. S. Geological Survey; L. L. Thatcher, U. S. Geological Survey; D. Welte, Pennsylvania State University.

¹ Appointment terminated September 30, 1957.

Fellows: R. G. Arnold, Princeton University; H. L. Barnes, Columbia University; L. A. Clark, McGill University; ² W. G. Ernst, Johns Hopkins University; N. Morimoto, Tokyo University; ³ P. M. Orville, Yale University; E. H. Roseboom, Jr., Harvard University; W. F. Schreyer, University of Munich; ⁴ J. R. Smith, Princeton University; ⁵ A. C. Turnock, Johns Hopkins University; ⁶ J. R. Vallentyne, Queen's University; ⁵ D. R. Wones, Massachusetts Institute of Technology.

Operating and Maintenance Staff

Office Manager: A. D. Singer.

Bookkeeper and Stenographer: E. T. Orozco.

Editor and Librarian: Miss D. M. Thomas.

Stenographer: Miss M. E. Imlay.

Typist: Miss G. C. Andrews.⁷

Stockroom Assistant: M. L. Kirby.

Mechanic's Helper: C. Baylor.

Janitor: M. Ferguson.

Chief Mechanician: F. A. Rowe.

Instrument Makers: J. F. Kocmaneck, O. R.

McClunin, G. E. Speicher, W. H. Suerth.⁸

Mechanic and Carpenter: E. J. Shipley.

Electrician: E. C. Huffaker.

Machinist: J. R. Thomas.

Apprentice-Machinist: W. R. Reed.

Building Engineer: R. L. Butler.

² Appointment from October 1, 1957.

³ Appointment from September 1, 1957.

⁴ Appointment from January 1, 1958.

⁵ Appointment terminated August 31, 1957.

⁶ Appointment from February 1, 1958.

⁷ Appointment from June 3, 1958.

⁸ Appointment from November 25, 1957.

DEPARTMENT OF PLANT BIOLOGY

Stanford, California

C. STACY FRENCH, *Director*

CONTENTS

	<i>page</i>
<i>Introduction</i>	263
<i>Personnel</i>	265
<i>Experimental Taxonomy</i>	266
Physiology of climatic races.....	266
Performance of <i>Mimulus</i> races and their hybrids in contrasting environments....	270
Studies on the interaction of temperature and light on <i>Mimulus</i> germination.....	272
<i>Poa</i> investigations	272
<i>Biochemical Investigations</i>	278
The absorption spectra of chlorophylls in various algae.....	278
Bleaching of chloroplast preparations by red light.....	286
Chlorophyll synthesis <i>in vivo</i> and <i>in vitro</i>	287
Quantum yield for the protochlorophyll-chlorophyll conversion.....	290
The polarization of fluorescence from the protochlorophyll holochrome.....	293
Reversible and irreversible changes of bacteriochlorophyll in chromatophores.....	295
Some properties of carotenoids in bacterial chromatophores.....	300
Germination of light-sensitive lettuce seeds in crossed gradients of temperature and light	303
<i>Bibliography</i>	304

INTRODUCTION

The nature and mode of action of chlorophyll as it occurs in plants, as contrasted with the properties of isolated chlorophyll, have continued to be major interests in the Department.

In the early part of the century the hope of the organic chemists was that determining the structure of isolated chlorophyll would make its mode of participation in photosynthesis evident. The structure was established long ago but led to no specific understanding of how chlorophyll functions.

Another promising line of attack was that of the biochemist. The extracted chlorophyll and the natural complex were considered to be related to each other as hemin is to hemoglobin or as a prosthetic group is to a complete enzyme. Moreover, biochemists had faith that isolation of functional units of chlorophyll and examination of their properties would greatly aid in understanding the action of chlorophyll. The pursuit of this idea, however, encountered preparative difficulties. Functional units of active chlorophyll with simple proportions of pigment and protein were not isolable from mature plants.

The freshly formed chlorophyll complex, as contrasted with that in mature plants, does, however, seem to be a definite chemical individual of the protein-prosthetic group type as established by Dr. Smith.

Within the more recent past Krasnovsky's photochemical reduction reaction of isolated chlorophyll has provided a more specific suggestion as to a cycle of chemical change that chlorophyll may undergo during photosynthesis.

In several laboratories the mode of action of the natural chlorophyll complex is now being described in terms of the theory of semiconductors. The present idea is to consider the functional units within a chloroplast as photocells that generate opposite electrical charges at different points. This viewpoint has already led to several

kinds of new experiments, and it provides a description of a possible mode of action in physical terms that are less specifically related to the chemical structure of the material involved.

And so the search continues not only to isolate the active chlorophyll complex but also to demonstrate by what means chlorophyll acts.

Whether chlorophyll acts in photosynthesis by going through a chemical cycle, or works through a physical process, the explanation of its very different properties in the natural complex and in pure form is still in the foreground. One basic problem, which may be resolvable experimentally, is to establish the range of variation of the measurable properties of the natural chlorophyll-*a* complex. How many recognizable forms of chlorophyll *a* exist in plants? Are there a large number of different forms scarcely distinguishable from one another, or are there only a few distinct types?

From the work of several different laboratories it is generally believed that two different natural forms of chlorophyll-*a* complexes occur together in most green plants. Neither of these two forms has been isolated as a chemical substance. Their existence is deduced from absorption spectra of plants and from the efficiency of photosynthesis at different wavelengths of light.

During the past year we have measured the derivative absorption spectra of chlorophyll in numerous algae and other plants. From the results of this survey it is thought that there must be more than two forms of natural chlorophyll *a*.

A comparison with the chlorophyll in the purple bacteria, which are capable of photosynthesis, is of particular value as a model in elucidating the forms of chlorophyll existing in green plants. Photosynthetic bacteria have a special kind of chlorophyll characterized by its absorption

band at 770 m μ on extraction with alcohol. In live bacteria this bacteriochlorophyll is combined with or adsorbed on proteins in several different ways to give various absorption bands *in vivo* that are sufficiently far apart in the spectrum to have been recognized long ago as different forms of the natural bacteriochlorophyll complex. The wavelength shift in the spectrum from that of *in vivo* to that of extracted bacteriochlorophyll bands is very much larger than the corresponding shifts of chlorophyll *a* in green plants. Since the shift is small in green plants, the possible difference between the various forms of the chlorophyll-*a* complexes is correspondingly small. Owing to the small difference of peak position in proportion to the width of these overlapping bands *in vivo*, the detection of the individual forms of chlorophyll *a* is more difficult than for those of bacteriochlorophyll.

When the various forms of chlorophyll *a* commonly present in green plants can be identified with certainty, and the factors controlling their formation and interconversions are well known, it may be possible to determine the relative ability of the different forms to carry on photosynthesis. One new form, very different from those previously recognized, has its absorption maximum at the very long wavelength of 695 m μ . This form is found in old but not in young cultures of the alga *Euglena*. Either old or young *Euglena* cultures give the usual chlorophyll when extracted with organic solvents. This 695-m μ pigment of *Euglena* therefore appears to be chlorophyll *a* combined in some unusual form of complex. The evidence for the presence of forms differing less in spectral absorption from one another is somewhat less direct than for this extreme example.

For many years the photochemical formation of chlorophyll in leaves from its precursor, protochlorophyll, has been studied by Dr. Smith and by investigators in several other laboratories. In spite of the great interest in this reaction, so important

to plant life, we know of no attempts to find out how much light is needed to form a molecule of chlorophyll.

Protochlorophyll with its carrier protein can now be removed from dark-grown leaves in an active form and purified to a reasonable degree. The absorption coefficients of protochlorophyll and of chlorophyll *a* are known within a few per cent. It therefore seemed feasible to measure the efficiency of the photochemical conversion of protochlorophyll to chlorophyll. For this determination the extracts were illuminated, and the amounts of the incident light absorbed by active protochlorophyll in the solutions were calculated. After exposure to light the amount of chlorophyll formed was measured spectroscopically. Yields ranging from about 0.5 to 0.7 (av. 0.6) molecule per quantum were found. A yield of 0.5 would mean that 2 quanta of light produce 1 molecule of chlorophyll. The experiments are being continued and the methods improved to obtain a more precise value.

Dr. Goedheer has investigated the reversible bleaching of the chlorophyll in several species of photosynthetic bacteria by chemical treatments and by light. The several coexisting forms of bacteriochlorophyll have been found to have different chemical reactivity. The form absorbing light at the longest wavelength (890 m μ) is the most active. This is the form that Dr. Duysens formerly found to receive the energy absorbed by the other pigments; it is also the fluorescent form. The purple photosynthetic bacteria with their different forms of chlorophyll *in vivo*, widely separated in the spectrum, are providing a more easily studied system with which the less advanced investigation on the various forms of chlorophyll *a* in green plants may be compared.

The emphasis in the study of plant relationships has progressed in recent years from the cytogenetic and transplant investigations to those in the area of comparative physiology. Why some plants thrive

in a particular environment and other, closely related, plants require a very different climate, even for survival, has become one of the main questions under study by the Experimental Taxonomy group.

Different species and strains of the monkey flower, *Mimulus*, have been found particularly suitable for comparison of physiological behavior, as measured in the laboratory, with their ability to grow in the contrasting environments of the three experimental gardens at different altitudes.

A number of climatic races of *Mimulus* plants are being grown at Stanford, Mather, and Timberline for laboratory studies of the way their rates of photosynthesis and respiration respond to variations of temperature and of light intensity. The recorded patterns of photosynthesis and respiration of these climatic races at known light and temperature conditions will be compared with the observed growth responses of the same plants at the three altitudes and in growth chambers in which temperature, light, and humidity will be controlled.

The rates of photosynthesis and respiration of cloned plants of *Mimulus* originally from diverse latitudes and altitudes have been explored in a preliminary survey. The results obtained to date show marked differences in rates of these basic functions for different plants. The interpretation of the differences in terms of climatic races, and their significance, if any, in natural selection in different environments, is an ultimate objective of these investigations.

In segregating progenies of the *Mimulus cardinalis-lewisii* cross the frequencies of certain flower colors varied greatly with the altitude at which the progeny had been

established. At high elevation, types resembling the alpine parent were predominant, while types resembling the lowland parent were favored in the milder climates. Apparently there is genetic linkage between floral characters and the physiological characters that determine survival at extreme conditions.

Studies have been started on the germination of seedling populations of contrasting parental and hybrid lines of *Mimulus* under crossed gradients of two controlled variables—temperature and light intensity. This technique is also being used to compare differences in germination capacity in races from contrasting habitats and their segregating F_2 hybrid progeny. In the latter, individuals that appear to differ in temperature and light requirements may be selected and subjected to further field and laboratory tests.

A major event of the past year is the completion of the cooperative experiments with the Agricultural Research Service outlined in Year Book 54, pp. 172-174, which were designed to test responses of apomictic strains of key parental and hybrid bluegrasses in a wide diversity of climates at experiment stations strategically located throughout the United States. The results emphasize a striking degree of climatic specificity of the apomictic strains. This is the first time that such a series of widely distinct parental species and their stabilized apomictic hybrid derivatives has been systematically studied over a wide range of climates on a nationwide basis, and these tests have therefore general biological as well as agronomic interest. The extensive data from these and other range grass investigations are being analyzed and summarized for publication.

PERSONNEL

Biochemical Investigations

Staff: C. Stacy French, *Director*, Harold W. Milner, James H. C. Smith
Visiting Investigators: Francis T. Haxo, Max Milner

Research Fellows: Joop C. Goedheer, Paul H. Latimer

Research Assistant: Jeanette S. Brown

Technical Assistant: William P. Fine

Clerical Assistant: Beverly Bogart

Experimental Taxonomy

Staff: Jens C. Clausen, *Emeritus*, William M. Hiesey

Visiting Investigator: John P. Decker

Research Assistant: Malcolm A. Nobs

Summer Research Assistants: Thomas R. Pray, David T. Mason

Technical Assistants: Ruth F. Elliott, Jennifer M. Wootton

Clerical Assistant: Marylee H. Eldredge

Gardener: Wesley B. Justice

Department Secretary

Wilbur A. Pestell

Mechanic

Richard W. Hart

Custodian

Richard P. Ludolph, Jr.

Dr. James H. C. Smith served as Councilor, Santa Clara Valley Section, American Chemical Society.

Dr. Jens C. Clausen served as a member of the Council of the Society for the Study of Evolution.

EXPERIMENTAL TAXONOMY

Physiology of Climatic Races

Harold W. Milner, William M. Hiesey, and
Malcolm A. Nobs

A critical evaluation of the physiological responses of climatic races of plants to changes in their external conditions is essential for an understanding of the mechanisms that make it possible for a race to thrive in one habitat and to fail in another, whereas another race may show opposite responses in the two habitats. Physiological characteristics undoubtedly have significance in the understanding of natural selection in different environments, a process now generally accepted as governing the direction of evolution. The unique advantages of races of the *Mimulus cardinalis-lewisii* complex for comparative physiological investigations have been reviewed previously. The *Mimulus* plants now under cultivation in connection with transplant and genetic investigations form a valuable reservoir of material for physiological studies. The combined approach, utilizing genetic and transplant studies in connection with physiological measurements as outlined last year (Year Book 56, p. 286), has been continued.

This year a survey was made of rates of photosynthesis and respiration of diverse climatic races of the *Mimulus cardinalis-lewisii* complex, over limited ranges of temperature, light intensity, and carbon

dioxide concentration. Young, vigorous, potted plants grown in soil in the greenhouse under approximately uniform but uncontrolled conditions were used for the measurements. Both cloned individuals grown from cuttings, and seedlings 2 to 3 months old, were used.

The upper part of the central stem, bearing two pairs of unfolded leaves, was sealed in the plant chamber; the rest of the intact plant remained outside. The lower enclosed pair of leaves was almost full size, but was still growing. The upper pair, about half size, was growing rapidly. For this reason, the total area of the two pairs of leaves was determined at the beginning and at the end of a series of experiments. An interpolated value of the leaf area was used for calculation of the rate of photosynthesis at each time of measurement. The increase in leaf area during an 8- to 12-hour experimental day ranged from 8 to 20 per cent of the initial area, usually being 12 to 16 per cent.

The leaf area enclosed in the apparatus was from 0.3 dm², the smallest that would give reliable measurements of photosynthesis and respiration, to 1.5 dm², with which CO₂ was assimilated almost as rapidly as could be measured accurately. When the size of the experimental plants permitted a choice, leaf areas between 0.5 and 1.0 dm² were taken.

Removal of CO_2 from the air in the apparatus by photosynthesis of the plant was proportional to time at concentrations from about 500 down to 300 parts per million (ppm). As the CO_2 became further depleted, the rate of its removal assumed an exponential form. Because of this, measurements of photosynthesis, which lasted 3 minutes, were started with 425 ppm CO_2 . Measurements of dark respiration were started with less than 300 ppm CO_2 and were continued until an accurately measurable change in CO_2 concentration occurred. The observed rate of photosynthesis or respiration in ppm CO_2/min was calculated to mg $\text{CO}_2/\text{dm}^2/\text{hr}$, the unit used by many workers.

Three 150-watt reflector spot lamps were arranged so that the illumination in the plane of the leaves was uniform within ± 5 per cent of the mean value of 3400 foot-candles (fc). Later, when need was found for a higher intensity, 300-watt lamps of the same type were arranged similarly to provide 11,000 fc at the leaf surface. A fan-cooled, 1.5-inch-deep water filter between the lamps and plant chamber removed most of the heat from the light beam. The temperature in the plant chamber was held constant within $\pm 0.1^\circ \text{C}$ by thermostatic controls. The light intensity incident on the leaves was varied by inserting calibrated screens between the lamps and the plant.

A Beckman infrared CO_2 analyzer was installed in the apparatus described in Year Book 56, pages 286–287. A Brown recorder was used to register continuously the CO_2 content of the air in the plant chamber. After calibration curves had been prepared for the analyzer, the operating characteristics of the analytical system were tested thoroughly. The response of *Mimulus* plants to different values of light intensity, temperature, and CO_2 concentration was then observed.

Replicate measurements of the photosynthetic and respiratory rates of a given plant specimen did not differ by more than ± 1 per cent. The change of photosynthetic

rate with temperature and light intensity was determined for several clone members propagated vegetatively from a single *Mimulus cardinalis* plant. Each clone member showed its maximum photosynthesis at the same temperature and light intensity. Under identical conditions the individual rates of photosynthesis of the clone members were the same within ± 3 per cent. The difference here may be due to slightly different stages of development of the various clone members, which were equal in age, but were not grown under controlled conditions before the measurements.

After the degree of reproducibility of measurements made on one plant, and on different members of one clone, had been established, a survey of the photosynthetic response of different races of the *Mimulus cardinalis-lewisii* complex was started. The rates of photosynthesis and respiration of each plant were measured over the same range of temperatures and light intensities. The temperatures selected were from 20° to 45°C in 5° steps. Light intensity was varied from 1500 to 5000 fc in seven roughly equal steps. Photosynthesis was measured three times under each set of conditions; then the dark respiration was determined at the same temperature. Values of photosynthesis were expressed as mg $\text{CO}_2/\text{dm}^2/\text{hr}$, and were corrected for dark respiration.

A preliminary survey was made on the 13 races of the *Mimulus cardinalis-lewisii* complex listed in table 1. All the plants were seedlings of similar age taken from large population samples; they represented plants originally from 13 locations ranging from near sea level to 10,500 feet altitude, and from 31° to 39°N . latitude. As each seedling may have individual characteristics that differentiate it from its siblings, it is assigned an individual number. In the survey 3 siblings of race 1 and 2 each of races 6 and 13 were used. Only 1 seedling each was taken in the other 10 races, making 17 seedlings in all. An effort was made to use plants as nearly in the same

physiological state of development as possible. The plants were in active vegetative growth and had not begun to set flower buds.

For each of the 17 seedlings the rate of photosynthesis was measured at several light intensities from 1500 to 3400 fc. At 20° C, 2 seedlings showed saturation at 2160 fc, 3 at 2450 to 2600 fc, 5 at 2800 fc, and 1 at 3200 fc. Only in the latter part of the work were 3 seedlings found that did not show light saturation below 3400 fc at 20°. At this point the more powerful light source was set up, and these 3 seedlings were found to become light saturated at 4000 to 5000 fc at 20°. The light intensity required for saturation at 33° was materially higher than that at 20°. Too few individuals were run at both temperatures to permit valid comparison of the relative saturating light intensities at the two temperatures. In future work the saturating light intensity will be determined at a temperature near the optimum for photosynthesis of the given plant.

Rates of photosynthesis at 20° to 45°, under high light intensity, were determined for each of the 17 seedlings of *Mimulus*. One seedling showed its maximum photosynthesis at 20°, 1 at 25°, 10 at 30°, 3 at 35°, and 2 at 40°. It is of interest that all 3 seedlings of race 1 (table 1) showed maximum photosynthesis at 30°. But the 2 seedlings of race 6 showed optimum photosynthesis at 20° and 35° respectively, and the 2 of race 13 had their optima at 30° and 40°. The optimum temperature for photosynthesis was measured at 3400 fc for all 17 seedlings, and at 5500 fc also for 5 of them. At the higher light intensity, the optimum temperature for photosynthesis remained the same although the rate in mg CO₂/dm²/hr was higher.

The photosynthetic rates of the 17 seedlings, determined for each one at its optimum temperature and 3400 fc, varied from 26.7 to 44.3 mg CO₂/dm²/hr. Values for the 10 seedlings of *M. cardinalis* ranged

from 26.7 to 38.2, and for the 7 seedlings of *M. lewisii* from 29.6 to 44.3. On the basis of these results, *M. lewisii* appears to have a statistically significant higher photosynthetic rate than *M. cardinalis*, although the sample is too small to justify this conclusion. Furthermore, the *M. lewisii* plants used in this work are probably a biased sample. They were the few individuals that grew to usable size from the survivors of large seedling populations grown in the Stanford environment.

The photosynthesis of each *Mimulus* seedling followed one of three general patterns in response to temperature. The curves, as shown in figure 1, are not to be

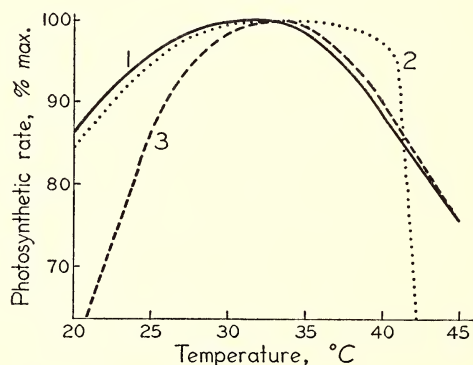


Fig. 1. Typical curves for response of photosynthesis to temperature in *Mimulus*.

taken as specific for individual plants. Rather, they indicate the different shapes of the observed response curves. The measured curve for any 1 of the 17 seedlings resembles one of the type curves more closely than it does either of the other two. Eight seedlings of *M. cardinalis* and 1 of *M. lewisii* showed maximum photosynthesis at 30° or 35° (except 1 at 20° and 1 at 25°), about equal activity at 20° and 40°, and a moderate drop between 40° and 45°, that is, a response having the general shape of curve 1. Four seedlings of *M. lewisii* had a response like curve 1 from 20° to 35°, but photosynthesis was considerably greater at 40° than at 20° (2 of these seedlings had their maximum at 40°), and there was an abrupt drop be-

tween 40° and 45°, indicated in general by curve 2. Two seedlings each of *M. cardinalis* and *M. lewisii*, indicated by curve 3, had definitely lower rates of photosynthesis between 20° and 30° than the others, but above 30° did not differ greatly in response from curve 1. The meaning of this empirical finding is not clear. Measurements of photosynthesis at close temperature intervals along the steep portions of response curves like 2 and 3 will be

nearly the same value for light saturation, and the three rates of photosynthesis under optimal conditions agree within ± 5 per cent of the mean. Thus, any 1 of these 3 seedlings might be a fairly good representative of the race. On the other hand, the 2 seedlings of race 13 have temperature optima at 35° and 40° and the same light saturation value, but their maximum rates of photosynthesis differ by 24 per cent. Again, the 2 seedlings of race 6 have tem-

TABLE 1. Original Habitats of *Mimulus* Races

Race	Culture	Location	Elevation, feet	North Latitude
<i>M. cardinalis</i>				
1	6546-5	Los Trancos Cr., San Mateo Co.,	150	37° 26'
	7113-8	California *	150	37 26
	7113-21		150	37 26
2	7114-6	Grass Valley, Nevada Co.	2,500	39 14
3	7115-15	Yosemite, Mariposa Co.	4,000	37 43
4	7117-4	Middle Fork, Tuolumne Co.	6,800	37 49
5	7118-9	Woodfords, Alpine Co.	5,500	38 47
6	7119-7	San Pedro Martir, Baja California	1,800	31 07
	7119-16		1,800	31 07
7	7120-15	San Antonio Pk., Los Angeles Co.	7,300	34 17
<i>M. lewisii</i>				
8	7121-1	Yosemite, Mariposa Co.	4,000	37 43
9	7122-13	Tamarack Flat, Mariposa Co.	6,300	37 47
10	7123-13	Smoky Jack, Mariposa Co.	8,700	37 49
11	7124-18	Porcupine Flat, Mariposa Co.	8,000	37 48
12	7125-2	Tenaya Lake, Mariposa Co.	8,300	37 50
13	7126-2	Timberline Sta., Mono Co.	10,500	37 58
	7126-8		10,500	37 58

* The habitats of all races except no. 6 are in California.

made in a search for significant differences between races.

No attempt is made now to link specific differences in photosynthetic response with definite races of *Mimulus*. It has been noted that measurements on a given plant specimen are highly reproducible, and that the values obtained for different members of the same clone are in very good agreement. But we have yet to establish how well or how poorly a single individual may represent a race. For example, the 3 seedlings of race 1, table 1, have the same optimum temperature for photosynthesis and

perature optima at 20° and 35°, require 4000 and 5000 fc for light saturation at 20°, and differ in maximum rates of photosynthesis by 18 per cent. Since the differences between the 2 seedlings of race 6 approach in magnitude the total range of differences observed, it is unsafe to say that the single seedlings measured in 10 of the races are fair representatives of their respective races.

Nevertheless, we have shown the existence of a range in photosynthetic response of different races of *Mimulus* that is far outside experimental error, and these dif-

ferences may well lead to characterization of races by their physiological responses. The next step will be to culture clones of a few of the most interesting seedlings measured, in order to make a more detailed and accurate study of the physiological differences between races.

In order to realize the greatest advantage from the precision possible in measurements of photosynthesis and respiration, it has become evident that it is essential to have as precise control as possible over all stages of growth of the plants before they are used for physiological measurements. Of even greater importance are measurements on identical clones grown under a wide range of controlled environmental conditions, to ascertain their entire pattern of response at different stages of growth. With this in mind, we have under construction a controlled growth chamber with a 30 by 30 by 32 inch space for the plants. In this chamber the light intensity, "day length" of illumination, temperature, and relative humidity will be the same for each plant, will be under automatic control, and will be variable over as wide a range as is needed to equal, or even to exceed, the range of these variables encountered in any natural environment.

Pending successful operation of the first controlled growth chamber, it is planned to construct more as the work develops. Two growth chambers would permit simultaneous comparison of the same clone under two sets of conditions, or of two clones under the same conditions. Additional units can be built as they can be utilized effectively.

Performance of Mimulus Races and Their Hybrids in Contrasting Environments

Malcolm A. Nobs and William M. Hiesey

The marked selective effect of different environments during critical stages of germination and early seedling establishment of *Mimulus cardinalis*, *M. lewisii*, and their F_2 progeny, reported in Year Book 56 (pp.

291-292), has been followed during the current year in experiments utilizing the contrasting climates of the transplant stations. Identical samples of the parental species and their hybrid were sown at the Stanford, Mather, and Timberline stations. The course of germination, establishment, and seedling elimination was followed at each station by studies on the expression and segregation of parental characters in surviving F_2 plants as they matured to flowering.

The inheritance of flower and vegetative characters in *M. cardinalis* \times *M. lewisii* appears, in general, to be controlled by a series of multiple genes, as indicated by segregations observed in F_2 and F_3 progenies grown at Stanford. There is, however, one striking character governing flower color that is controlled by a single pair of genes (cf. Year Book 56, p. 291). A dominant gene carried by *M. lewisii* of high altitudes suppresses the formation of yellow chromoplasts in the upper epidermis of the petals of segregating F_2 progeny, and when present allows only various polygenic expressions of varying shades of pink to appear in the flowers. In plants lacking this dominant gene, as in *M. cardinalis* of the lowlands, yellow chromoplasts are formed. Second-generation hybrid progeny between *cardinalis* and *lewisii* that likewise lack this gene display a structural pattern of pink and yellow pigments that is expressed in various shades of color, ranging from orange to vermilion. This pair of genes appears to be linked genetically with other morphological characters affecting flower structure, and likewise with the capacity of individual progeny to survive in the contrasting environments at Stanford, Mather, and Timberline.

Table 2 shows the shifts in segregation ratios of pink- to orange-flowered F_2 progeny surviving at the three transplant stations. The differences in ratio are clearly significant: *lewisii*-like pink-flowered types survive in far greater proportion

among the seedling progeny germinated and established at Timberline than among those germinated and established at Stanford. At Mather the proportion is intermediate, but more like that of the Stanford than the Timberline plants. Likewise, at Stanford there is a strikingly significant difference between F_2 progeny sown and established during winter as compared with those sown during summer: the winter-sown plants, which germinate and become established under cooler temperatures, show a much higher proportion of pink-flowered types among the surviving progeny, as described in Year Book 56.

Similar tests of seedling establishment at the transplant stations were made con-

species, both of which fail to survive in this environment. For *M. cardinalis* from the coast, the winters at Mather appear to be too severe, whereas, for *M. lewisii* from near Timberline, the summers are too hot and too long. The F_1 hybrid, however, succeeds well at Mather (cf. Year Book 52, p. 182). The F_2 progeny displays not only a high percentage of survival but also a very considerable amount of vigor, equal to, or often exceeding, that of the F_1 . It is apparent that the F_2 progeny has inherited complementary physiological characteristics from the diverse parents which make it possible for it to succeed at Mather. Also noteworthy is the fact that, among the F_2 progenies established at the three

TABLE 2. Segregation among F_2 Progeny of *Mimulus cardinalis* \times *M. lewisii* Germinated and Established at Three Altitudes

Conditions of Germination and Early Establishment	Per Cent Seedling Mortality	No. of Plants Maturing	Ratio of Pink- to Orange-Flowered Individuals
At Stanford, summer sown	89	180	1.7 : 1.0
At Stanford, winter sown (elevation, 150 ft)	71	463	4.4 : 1.0
At Mather, summer sown (elevation, 4600 ft)	71	104	2.5 : 1.0
At Timberline, summer sown (elevation, 10,000 ft)	78	242	8.7 : 1.0

currently with the parental species, coastal *M. cardinalis* and subalpine *M. lewisii*. After wintering at Timberline, there was no survival of seedlings of *M. cardinalis* and 40 per cent survival of *M. lewisii*; at Stanford, on the other hand, there was 90 per cent survival of *M. cardinalis* and only 5 per cent of *M. lewisii*. All the evidence points, therefore, to a marked selective elimination of *Mimulus* seedlings at the three transplant stations that is strongly correlated with genetically determined flower and other morphological characters. Also, the direction of selection in the second-generation hybrids is the same as that expressed in the characters of the parental races.

A notable feature of the F_2 progeny germinated and established at Mather is its outstanding vigor at this mid-altitude station as compared with the parental

transplant stations, only at Timberline were survivors found that had an association of characteristics closely resembling those of the subalpine *M. lewisii*. Plants resembling *M. cardinalis* were present in progenies grown at all three stations, but at Stanford they were of much higher frequency than at Timberline.

The individual plants of the F_2 progenies germinated and established at Stanford and at Timberline are being cloned and grown as transplants at Stanford, Mather, and Timberline in order to determine with certainty, over a period of years, the extent to which morphological characters are correlated with physiological responses. These plantings will also provide critical plant material for detailed laboratory studies on the comparative physiology of selected individual plants. The coordination of the genetic and trans-

plant studies with the laboratory investigations should lead to a well rounded picture of the physiological structure of ecological races in relation to mechanisms of natural selection as they operate under approximately natural conditions.

Studies on the Interaction of Temperature and Light on Mimulus Germination

Ruth F. Elliott

In the lettuce seed experiments described on pages 303–304, the crossed-gradient principle was found to be applicable to studies of seed germination. Therefore a start has been made in studying the effect of light and temperature on the germination and early seedling growth of parental and hybrid strains of *Mimulus*. Since only a few experiments have been conducted, the results given are somewhat tentative.

For this work the apparatus (Year Book 55, pp. 261–265) for growing microscopic algae has been used with only minor modification. This apparatus has a 12 by 11 inch shallow growth chamber with a constant temperature gradient from left to right and a constant light intensity gradient from front to back. Two layers of filter paper covered with a piece of black cloth are spread over the growth chamber, and the seeds to be tested are spaced as evenly as possible on the moistened cloth. At the back of the chamber a narrow strip an inch wide is covered with a special metal cover so that these seeds remain in total darkness, but still in the temperature gradient. The seeds are left to germinate and develop in the crossed gradient for about a month. After germination the seedlings are sprayed with nutrient. Daily photographs of the chamber are taken to provide a record of the progressive changes.

So far two strains, one of *M. cardinalis* and one of *M. lewisii*, have been studied. Both begin to germinate after 3 days, and the germination continues for 9 days. In *M. cardinalis* germination begins at the higher temperatures (approximately 26°

to 33° C) and moves down to approximately 14° C. In *M. lewisii* germination begins at approximately the same temperature range as in *M. cardinalis* and then moves both up to about 36° C and down to about 12° C. The experiments show that germination in both strains is very sensitive to temperature, each strain having a distinct range above and below which it will not germinate. For *M. cardinalis* this range is about 14° to 25° C; *M. lewisii* has a wider tolerance, 12° to 36° C. The rate of germination also varies with the temperature, and a different pattern is shown by the two strains. There is little individual variation among the seeds of a single strain, most seeds germinating where any germination takes place.

The light intensity used (25 to 900 fc) had no differential effect upon the germination of the seeds of either strain, but the seeds of *M. cardinalis* kept in the dark do not germinate in the temperature range (8° to 38° C). On the other hand, *M. lewisii* germinates equally well in the dark and in the light. The details of the light requirement in *M. cardinalis* have still to be determined. Previous workers have found germination to be light-sensitive in two other species of *Mimulus* (*M. ringens* L. and *M. luteus* L.).

Thus there appear to be interesting differences between the germination requirements of the two parental strains of *Mimulus* studied. Their temperature tolerance, the rate of germination at different temperatures, and their sensitivity to light seem to be characteristic for each strain. In the future work, it is hoped to study segregating F₂ hybrid progeny in this way. Individuals of a population having different temperature and light requirements could be selected from the growth chamber for field and laboratory tests.

Poa Investigations

Jens Clausen, William M. Hiesey, and
Malcolm A. Nobs

As the *Poa* program approaches completion, it is helpful to review the various

phases of the investigations that were necessary for understanding the broad relationships between species, the probable evolutionary history within this remarkable genus, and the agronomic potential for synthesizing constant new interspecific hybrids. These phases have been:

1. The production of hybrids between apomictic members of distinct taxonomic sections of the genus.

2. Progeny tests of hybrids, leading to the development of new apomictic strains within the genus that combine the hereditaries of highly distinct species.

3. Exploration of the evolutionary mechanisms by which new constant forms evolve in this primarily apomictic genus.

4. Determination of the tolerance ranges and patterns of growth response under field conditions of new apomictic lines as compared with the parents at contrasting altitudes, latitudes, and longitudes, and in controlled environments.

5. Determination of the breeding habits of experimental apomicts, and comparison with apomicts of well established *Poa* species.

6. Formulation of concepts of the evolutionary mechanisms and history of groups of species within the genus, especially within the highly adaptable species complex of *Poa pratensis*.

Ranges of environmental tolerance in apomictic hybrids. During the past year special emphasis has been placed on phases 4 and 5. The *Poa* plantings that were established in 1955 in collaboration with the United States Agricultural Research Service and Soil Conservation Service (Year Book 55, pp. 236-238) have now matured. It was therefore possible to obtain comparable numerical data on the performance of the same apomictic lines of *Poa* grown at experiment stations across the United States. During late May to early July 1958, these plantings were visited and studied by Clausen and Nobs. The resulting information is being pooled with the data from the altitudinal transplant

stations in California and with previous information from other environments.

The stations visited were located as follows:

Within the Atlantic drainage system:

Blacksburg, Allegheny Mountains, Virginia, at 80° 20' W. longitude, 37° 30' N. latitude, and 2300 ft altitude.

Within the Mississippi-Missouri drainage system:

Lexington, Kentucky, at 84° 30' W., 38° 05' N., and 970 ft altitude.

Purdue University, Lafayette, Indiana, at 86° 58' W., 40° 25' N., and 700 ft altitude.

Columbia, Missouri, at 92° 25' W., 39° 00' N., and 800 ft altitude.

Rosemount, near St. Paul, Minnesota, at 92° 58' W., 45° 00' N., and 800 ft altitude.

Moccasin, Judith Basin, Montana, at 109° 25' W., 47° 30' N., and 4250 ft altitude.

Within the Snake-Columbia River drainage system:

Tetonia, Teton Basin, Idaho, at 111° 15' W., 43° 40' N., and 6200 ft altitude.

Pullman, Palouse Prairie, Washington, at 117° 10' W., 46° 50' N., and 2300 ft altitude.

Within California:

Hall's Flat, N. of Susanville, Lassen County, Northern Great Basin, at 121° 18' W., 40° 45' N., at 6200 ft altitude.

Stanford, Santa Clara Valley, at 122° 13' W., 37° 26' N., and 100 ft altitude.

Mather, west slope of Sierra Nevada, at 119° 55' W., 37° 53' N., and 4600 ft altitude.

Timberline, east slope of Sierra Nevada, at 119° 05' W., 37° 57' N., and 10,000 ft altitude.

It had not previously been possible to conduct systematic transplant experiments of identical clones over an environmental range of this magnitude. The use of apomictic seed clones in *Poa* has made this practicable. The genetic identity of the approximately 40 apomictic strains could be safely verified at all the stations. The parent species are highly distinct and

from contrasting native habitats, and their apomictic hybrids have identifiable characteristics.

The uniformity of the 40 clones among replications within each station was in sharp contrast with conspicuous differences in phenotypic expression of the same strains in the different environments. The morphological distinctness between the apomicts, however, was recognizable in each environment. Some clones were modified considerably from station to station in size, leafiness, degree of flowering, and length of rhizomes, whereas others having more highly buffered ranges of physiological tolerance were of approximately the same vigor and size everywhere.

Poas of local origin invaded the test plots at certain stations, and even some of the strains being tested had, during their third year, been able to sow themselves and invade adjacent plots at stations where they were vigorous. At any one station, most of the volunteers were among the strains showing the greatest vigor there, and these frequently overwhelmed the weaker strains being tested, which could sometimes be found only with considerable difficulty.

Three strains that originally were thought to be apomictic because of their apparent uniformity in one environment varied highly in other environments, proving that they were sexual. Like some commercial strains, they were composed of mixtures of biotypes.

The relative vigor of the hybrids as compared with the parental strains changes from place to place. Environment is, accordingly, a major factor in assessing hybrid vigor. The ranges of tolerance of the parental species differ greatly, and the ranges of the hybrids differ from those of their parents.

Poa scabrella, the bluegrass of the California Coast Ranges and foothills, for example, has a narrow range of tolerance. It is limited to the Mediterranean-type winter-rain region, and was unable to sur-

vive even in the southern states of Louisiana, Kentucky, and Virginia. *Poa ampla*, the big bluegrass of the Palouse Prairie, is highly successful in the Pacific Northwest, including Oregon, Washington, and Idaho. It survives at low to moderate altitudes in California and Montana, but the survival rate was low in the central states and Virginia.

The apomictic biotypes of *Poa pratensis*, Kentucky bluegrass, are far more tolerant to wide ranges of environment than those of the two species mentioned above. They differ also from most wild plants in being highly adjustable and in showing only slight degrees of modification. *Poa pratensis* nevertheless contains certain ecotypes that have a narrow range of tolerance. An example is the alpine race from near Timberline, which dies at mid-altitudes, can be kept alive only for a year or two at Stanford, and survives for but a slightly longer period in the ecologically intermediate environment at Pullman. *Poa pratensis alpigena* is an example of an ecotype subspecies, native to a region bordering both sides of the Arctic Circle, that survives, but is weak and unsuccessful, farther south.

Other strains of *Poa pratensis*, such as Athabaska from the parklands of central Canada, the coastal Newport strain from Oregon, the mid-altitude apomict from Mather on the west slope of the Sierra Nevada, and the Leevining strain from the desert plateau east of the Sierra Nevada, all have a high degree of tolerance to differences in environment. In general, they survive and compete fairly well in the western, central, and eastern sectors of the United States, and likewise even at fairly high latitudes in western Europe. This range of tolerance far exceeds that of the bunch grass *Poa* species from western North America.

The apomictic hybrid lines were derived from plants selected for their outstanding vigor in experimental plots at Pullman, Washington, or in the milder climate at

Stanford, both far western environments. In the bluegrass region of the central states, local strains of *Poa pratensis* resulting from long selection frequently invaded the experimental plots in strong competition with the hybrid lines. Some of the hybrids, however, were able to compete successfully with the local strains.

Apomictic hybrids combining the hereditaries of *Poa ampla* from Kahlotus, Washington, a poor survivor in the central states, and of the Athabaska strain of *Poa pratensis* which thrives in this area, were in this category. Four apomicts of this combination, each distinct from the others, yet all taxonomically classifiable as *Poa pratensis*, were especially noteworthy. Two or three of these hybrid apomicts have vigor comparable with that of local volunteer *Poa pratensis* in the midwest. They are also among the most successful strains in the Pacific Northwest, and likewise perform well in a wide range of other environments.

In contrast with these, an *ampla-pratensis* combination of a different parentage is far more specialized. The parents were *Poa ampla* from Albion, Washington, and *Poa pratensis* from Mather in the central Sierra Nevada. The apomictic lines of this hybrid were selected for their outstanding vigor at Pullman, but they proved to be weaker than either parent in the central states. From Pullman eastward, the Albion-Mather hybrids and their *ampla* parent gradually show decreased adaptiveness.

When *pratensis alpigena* from Lapland is substituted for *pratensis* from Mather as the male parent in the above cross with *ampla*, the range of tolerance of the resulting apomictic lines increases, although the *alpigena* parent itself is more poorly adapted to low latitudes than Mather *pratensis*. The *ampla-alpigena* hybrids were especially successful at Rosemount, Minnesota. Here the *ampla* parent is a nonsurvivor, and the *pratensis-alpigena* parent grows only weakly, but the hybrids are in a class with local forms of *Poa pratensis*.

Other tolerant apomicts include two hybrids between *Poa scabrella* and *P. pratensis*. Their *scabrella* parent is unable to survive for any length of time outside the lower altitudes in California. Morphologically the hybrids would be classified as forms of *Poa pratensis*, although the *scabrella* genome has contributed characteristics that make them physiologically distinct.

The complexity of the various environments as reflected in the performance of the cloned apomicts is illustrated by the responses of two tolerant biotypes. One is a spontaneous hybrid of *Poa ampla* from Condon, Oregon, pollinated by an unknown form of *Poa pratensis* that gave rise to an F_2 segregant which is apomictic. The apomict is vigorous not only in the Palouse Prairie region but also in the Coast Ranges and valleys of California, where it is winter-active. At the higher altitudes of Mather and Timberline it and its *ampla* parent are both weak. It was therefore anticipated that the hybrid would be weak in regions having cold winters. Contrary to expectation, it was able to compete fairly successfully with the local strains in the central states, although the *ampla* parent is unsuccessful there. At Tetonia, Idaho, on the other hand, the *ampla* parent is exceedingly vigorous but the hybrid is highly unsuccessful. The Tetonia habitat shares high altitude with Mather and Timberline, but the *ampla* parent responds differently in the high altitudes of Idaho and California.

Among other biotypes that illustrate the complexity between environments is an apomictic clone of *Poa pratensis* from a maritime habitat at Newport, Oregon. This strain is usually tolerant, inasmuch as it will survive with vigor at 10,000 feet altitude at Timberline, is able to grow successfully north of the Arctic Circle in Swedish Lapland, is highly tolerant to the environments of the central states and Virginia, and was the only *Poa* to produce creditable growth as far south as Franklinton, Louisiana, at 30° N. At Moccasin,

Montana, however, this strain failed; except for single survivors, it has been almost completely eliminated from three replicated plots.

Moccasin has severe winters; nevertheless, one of the strains surviving there was thought to be especially nontolerant of cold winters, the apomictic line 4569-1 of *Poa scabrella* × *ampla*. This line barely survives the winters at Mather and dies at Timberline, and likewise in the central and eastern states, and throughout Scandinavia.

These apparently contradictory responses suggest that the survival of a biotype depends on the balance between many environmental factors, and that any one factor can become limiting even if all the other essential conditions are satisfied. Winter survival, for example, depends not only on the ability to survive cold temperature but also on the capacity of the plant to produce sufficient food during the growing season to carry it over the winter. At Pullman the 4569-1 line is killed when unusually mild periods occur in midwinter that induce premature growth at the expense of stored food reserves. Perhaps the nonsurvival of 4569-1 in most environments is not due to low temperatures so much as to its low-temperature threshold for breaking winter dormancy.

Such observations emphasize the intricate balance between heredity and environment in determining the success of plants. This balance makes it necessary for the plant breeder to develop strains suitable for specific areas. Some biotypes, however, appear to be physiologically buffered so as to tolerate highly diverse environments. They may be produced by combining the heredities from races of contrasting environments, a situation that has apparently been realized in some of the hybrids such as *Poa ampla* × *P. pratensis* of the Kahlotus-Athabaska series, and the Albion-Lapland combinations.

Apomicts of quadruple hybrids. In 1951 several hybrids were intercrossed

(Year Book 51, pp. 113, 115-117), and a fair number of quadruple combinations were obtained. One of them was the hybrid between 4711-3, *Poa scabrella-pratensis*, and 4683-1, *Poa ampla-alpigena*, producing the quadruple combination *Poa scabrella-pratensis-ampla-alpigena*. Both the primary apomictic hybrids, 4711-3 and 4683-1, were selected in the F₂ generation after moderate recombination of the parental heredities had taken place.

The primary hybrids behave very much like highly distinct strains of natural species of *Poa*: they differ morphologically from each other and from other Poas, and they are constant and fertile. These criteria indicate that they qualify as stabilized and distinct apomictic strains of the *Poa pratensis* complex.

The quadruple F₁ hybrids have morphological characters of four parental species, namely *Poa scabrella* from Las Posas of the mild southern California coast range, *Poa pratensis* from Mather, *Poa ampla* from Albion, and *Poa pratensis* ssp. *alpigena* from Swedish Lapland. The four parents therefore are highly diverse taxonomically, ecologically, and geographically.

Sample F₁ plants of this quadruple hybrid were planted at the three transplant stations in 1953, and most have survived to the present writing, indicating a wide degree of tolerance. Seeds from open pollination of six F₁ plants were planted at Stanford in 1957, and in the spring of 1958 they reached flowering stages. One of the progenies from the F₁ plant 6310 is highly apomictic despite its highly mixed parentage, and two others are moderately apomictic. Three progenies are sexual, showing complex segregations.

The apomicts and sexuals of this quadruple combination are similar to those obtained in the primary hybridizations. There are the usual aberrant plants in the apomictic strains, and the percentage varies from progeny to progeny. The sexuals are highly variable, although no more so than in the primary crosses; the tendency to

absorb pollen from additional species for further hybridization remains, as with other normal sexuals. It is therefore apparent that the limit of absorbing heredities from various species in this versatile genus has not yet been reached, but can probably continue for geologic periods of time.

Hybrids between nonapomictic species of Poa. Besides its many species north of the equator, the genus *Poa* has considerable concentration of species in temperate South America and in Australia and New Zealand. Apart from a few montane species in the South American Andes, the *Poas* of the southern hemisphere are geographically well separated from those in the northern by the broad tropical belt on both sides of the equator where the genus does not occur.

Species of *Poa* occurring in Argentina and in southern Brazil are grouped in the subgenus *Dioicapoa* and are dioecious, having male and female plants, whereas most *Poas* are hermaphroditic. Several of the species studied by Argentinian cytologists, and one by us, are tetraploid, having $2n=28$ chromosomes. This fact, and the dioecious character, suggest that the group is predominately sexual. Some of the North American dioecious *Poa* species resemble the South American in their inflorescence characters, suggesting that they belong to the subgenus *Dioicapoa*. Among these are a tetraploid complex of dune species along the Pacific coast, *Poa macrantha* and *P. douglasii*, and an octoploid species of the southern Great Plains, *Poa arachnifera*, or Texas bluegrass.

The Australian and New Zealand species comprise another distinctive group, but their taxonomic relationships to the other *Poa* species are not yet clear. This group includes tussock forms that cluster around the New Zealand species *Poa caespitosa* and its near relatives in Australia. A lowland form of the New Zealand species is highly polyploid, having approximately 100 chromosomes, whereas an Australian lowland form has $2n=56$ and is sexual (Year Book 53, p. 157).

Using the Australian form of *Poa caespitosa* and a strain of *Poa arachnifera* from Stillwater, Oklahoma, to represent these groups, a few exploratory crossings were made in 1951 (Year Book 51, pp. 112-113). The parental species and the F_1 hybrids survive at Stanford. They were planted at the mountain stations in 1953, but *caespitosa* and its hybrids proved to be generally unadapted for the more severe climates in the Sierra Nevada. Some F_2 progenies planted at Stanford flowered for the first time in 1958.

Three F_1 hybrid combinations of the Australian *Poa caespitosa* succeeded. One of them, *Poa pratensis*, Mather \times *P. caespitosa*, Canberra ($2n=68$ and 56 , respectively), is completely sterile. Another combination, *Poa compressa*, Crescent Mills \times *P. caespitosa*, Canberra ($2n=42$ and 56), yielded a vigorous and moderately fertile F_1 , but the F_2 population obtained by open pollination of the F_1 is exceedingly weak. A sample of 90 F_2 plants was set in the spring of 1957, but only 13 remained alive up to June 1958; the culture is highly variable, and none of the F_2 plants have approached the parental species, or the F_1 , in vigor. Both these hybrids of *Poa caespitosa* with two of the genetically most highly buffered *Poa* species of the northern hemisphere therefore proved to be incapable of genetic interchange in the F_2 and subsequent generations.

The third combination, *Poa caespitosa*, Canberra \times *P. arachnifera*, Stillwater ($2n=56$ and 56), produced several F_1 's that are moderately vigorous at Stanford, whereas clones transplanted to the mountain stations soon died. The F_1 's are fully fertile, however, and three F_2 's composed of 90 plants each were planted at Stanford, resulting in cultures in which there are 41, 51, and 77 survivors, respectively. The F_2 culture with the 77 survivors has excellent vigor, a surprising fact considering the diverse parentage. The morphological influence from *Poa caespitosa* is prevalent in this and the other hybrid progeny of

caespitosa, although that influence varies in degree from plant to plant among the F_2 . It is therefore obvious that the hybrid is sexual. Both parents are highly polyploid, and the segregations suggest that the chromosomes of the two parent species predominately pair among themselves, although occasionally interchanges between the *caespitosa* and the *arachnifera* heredities occur.

The ancestors of *Poa caespitosa* and *P. arachnifera* must have been separated for long geological periods; one is hermaphroditic and the other dioecious. Their heredities are, nevertheless, sufficiently compatible to permit them to intercross and also to reproduce a hybrid population that is fairly distinct from all other Poas. It is

therefore obvious that not only the apomictic but also some sexual species of *Poa* have unusual ability to combine their heredities.

Preparation toward publication. During the year Miss Jennifer Wootton, a graduate student in biology at Stanford from the University of Reading, England, worked on statistical tabulations of data of *Poa* populations grown over a period of years at Pullman and other localities. This is a beginning step in the preparation of a published survey of the *Poa* investigations. Miss Wootton also has made drawings showing critical taxonomic characters that distinguish several of the *Poa* species and their hybrids, some of which will be incorporated in the final report.

BIOCHEMICAL INVESTIGATIONS

The Absorption Spectra of Chlorophylls in Various Algae

C. S. French and Ruth F. Elliott

The absorption spectra of live leaves and of algae have been measured by many investigators for a number of different purposes. All the measurements have shown the absorption peaks of chlorophyll *a* to be broader and at longer wavelengths in the natural state than the corresponding bands of pure chlorophyll in organic solvents. This shift of peak position on the wavelength scale is taken to mean that the chlorophyll is in some way combined with or adsorbed on other cellular components, presumably proteins.

The amount of this wavelength shift is well known to differ from one plant to another. Shibata observed forms of chlorophyll *a* having peak positions at about 670 and about 684 m μ in dark-grown plants at appropriate times after exposure to light. Krasnovsky has interpreted the variability of peak positions found in plants as being due to different proportions of a short-wavelength form of chlorophyll C670 and a long-wavelength form C680.

Studies of the shape of the red absorp-

tion band of chlorophyll in plants give much more information than can be obtained from considering only the peak position. For studying the shapes of curves derivatives are particularly valuable. Small differences in the shape of similar absorbance curves show up much more strikingly in the first derivative curves than in the absorbance curves themselves. A spectrophotometer for recording directly the first derivative of absorbance with respect to wavelength was described in last year's report.

With the derivative spectrophotometer we have measured the spectra of more than 50 species of plants, mostly algae. The derivative curves obtained have led to the qualitative conclusion that two or more forms of chlorophyll *a* are present in most species, but the quantitative analysis of the data is still in its early stages.

A primary objective of this survey of the derivative absorption spectra of algae is to define the various chlorophyll-*a* components *in vivo* by means of their individual spectra. In many algal spectra distinctive absorption by chlorophylls *b* and *c* is evident in addition to the main chlorophyll-*a* bands. The parts of the curves

attributed to chlorophylls other than *a* will be discussed first. Most of the spectra have been measured only from 600 to 750 m μ to avoid the region of carotenoid absorption. The complications due to numerous carotenoids make the blue and green regions difficult to analyze. A few algae, however, were measured at shorter wavelengths to show the derivative spectra of phycobilin pigments.

Pure pigments. To identify the contributions of chlorophylls *a*, *b*, *c*, and *d* to the derivative spectra of living cells it is convenient to have curves for the isolated pigments even though the wavelength positions and widths are different from those of chlorophylls *in vivo*. Derivative spectra are given in figure 2 for *a* in acetone and for *a* and *b* in ether. The region of low absorption of ether solutions is repeated on a scale enlarged about 10 \times . The curves of figure 2 for pure chlorophyll *a* in acetone and in acetone-water mixtures are useful for determining whether other chlorophylls than *a* are present in extracts of algae.

The derivative curve for the main red band of both chlorophylls *a* and *b* in ether is very nearly symmetrical except for a small amount of overlap by the small 615-m μ band. In the live algae, however, this symmetry is not usual. Derivative spectra of chlorophylls *c* and *d* have not yet been measured in solutions except for a small amount of chlorophyll *d* in the presence of much more chlorophyll *a*, as illustrated later in figure 5.

Algae with only chlorophyll *a*. We have measured the spectra of many algae that show absorption only by chlorophyll *a* in the 600- to 700-m μ region. Some red and blue-green algae, although lacking chlorophylls other than *a*, do have complications in this region from phycocyanin absorption. Figure 3 gives some derivative spectra of algae whose absorptions are due almost entirely to chlorophyll *a*. The curve for *Callithamnion*, a red alga, has the simplest shape of all, and that for *Ochromonas*

the most complicated. The minor inflections on the steep portion of the *Ochromonas* curve are reproducible and well outside experimental error. The curves for *Vischeria*, *Botrydiopsis*, and other species with only chlorophyll *a* are intermediate in complexity. Attempts to fit such spectra by adding together curves representing the spectra of two assumed components have not been successful, indicating that more than two components may be present in many algae.

Algae with chlorophylls *a* and *b*. Green algae have chlorophyll *b* as well as *a*. A few spectra of green algae are given in figure 4. *Haematococcus* has a larger chlorophyll *b* peak in proportion to chlorophyll *a* than any other alga studied.

The derivative curve of a single absorption band has a positive region on the short-wavelength side of the peak and a negative region on the long-wavelength side. The negative component of the chlorophyll-*b* derivative curve overlaps part of the positive region of the chlorophyll-*a* derivative curve. Therefore the algae with chlorophyll *b* have a partly distorted chlorophyll-*a* spectrum. Nevertheless, the region of chlorophyll-*a* absorption at wavelengths longer than about 670 m μ shows, by its diversity in different species, that algae with chlorophyll *b*, as well as those with only *a*, have different forms of chlorophyll *a in vivo*. Curves for *Carteria* and *Coccomyxa* were included in figure 4 to illustrate the variation in the negative branch of the derivative spectrum.

The chlorophyll-*b* region of *Ulva* is particularly interesting in showing a double chlorophyll-*b* peak at 640 to 650 m μ . It is not yet known whether this doubling is due to a different form of chlorophyll *b in vivo*, to a different extractable chlorophyll, or to absorption by a nonchlorophyllous pigment. The double peak was found to varying degrees in the three specimens of *Ulva* investigated.

The shoulder at about 675 m μ on the *Chlorella* curves in figure 4 is attributed

to the presence of two chlorophyll-*a* forms *in vivo* that are farther separated in wavelength than the components of many other algae. Lowering the temperature to 6° C

in *Chlorella* may be due to a third component, intermediate in wavelength position, filling the gap between the two widely separated components of *Chlorella*.

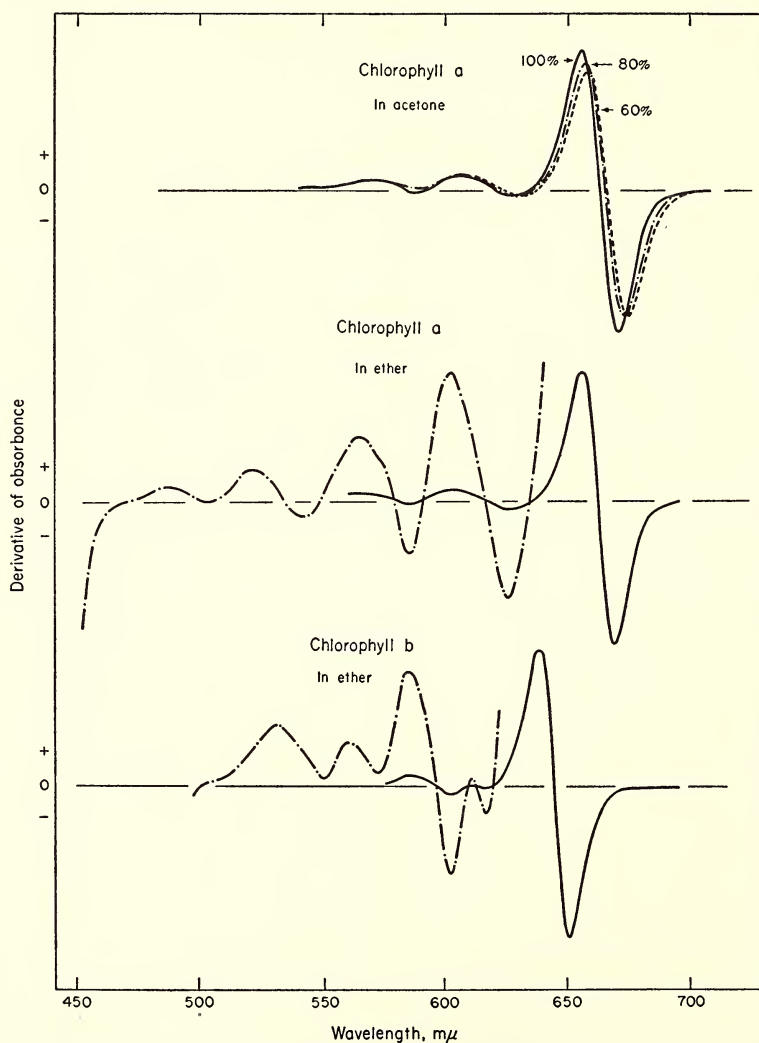


Fig. 2. Derivative spectra of isolated chlorophylls *a* and *b*. Upper: chlorophyll *a* dissolved in 100, 80, and 60 per cent acetone. Middle: chlorophyll *a* in ether. Lower: chlorophyll *b* in ether, corrected in the 645–680 mμ region for ca. 10 per cent contamination by chlorophyll *a*. The absorbance scales for the dot-dash lines are about 10× those for the continuous lines.

during the measurement makes the shoulder sharpen to a minor peak. This effect is attributed to the small influence of temperature in narrowing the bands of the individual components. The absence in other algae of this distinct shoulder seen

Algae with chlorophylls a and c. Curves for brown algae and diatoms that contain chlorophyll *c* show a characteristic shape in the region from 600 to 640 mμ, best seen in the *Navicula* curve of figure 5, although the spectrum of *Cryptomonas* is

perhaps more typical of organisms with chlorophyll *c*. The absorption of chlorophyll *c* is far enough away to cause no

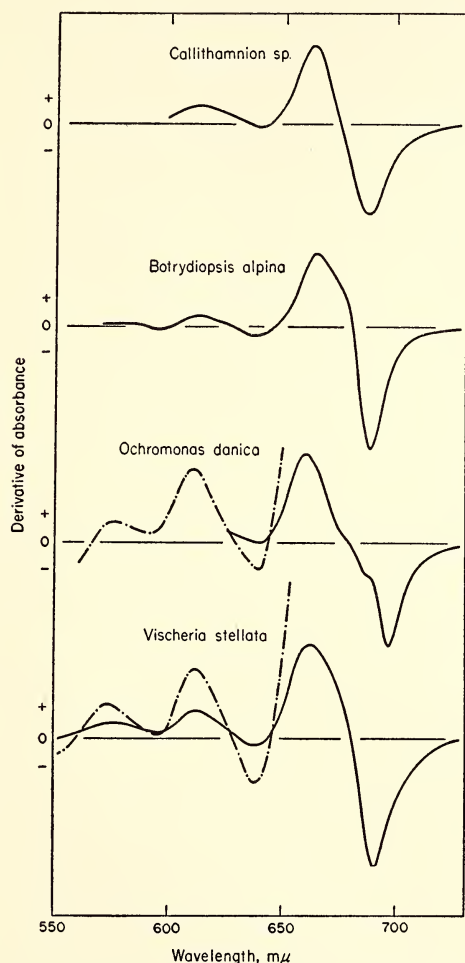


Fig. 3. Derivative spectra of algae containing no other chlorophylls than *a*. The variation in shape between the different spectra is attributed to the presence of several different forms of chlorophyll *a* *in vivo* in different proportions. The region of minor bands is also given on enlarged scales for two of the algae. We are indebted to Dr. M. B. Allen for *Ochromonas* and *Callithamnion*. The *Callithamnion* was ground in glycerine because of its unfavorable shape for direct measurement.

appreciable distortion in the main peak of chlorophyll *a*.

Algae with chlorophylls a and d. The absorption of chlorophyll *d* in an intact

alga has not yet been detected. A few experiments for this purpose were made with Dr. Jeanette S. Brown. One sample each of *Gigartina papillata*, *Endocladia muricata*, and *Gloiophloeia confusa* ground in phosphate buffer, pH 8.6, with glycerine gave a shoulder at 720 mμ, as in figure 5 for *Gigartina*, when it was measured with high amplification. However, no trace of a chlorophyll-*d* component was found in similar buffer-glycerine extracts of other samples of these red algae and of several other species. The curve measured at high amplification for three layers of the red alga *Iridophycus* shown in figure 5 is typical of these negative results.

Derivative spectra of methanol extracts (2-min extraction) containing both chlorophylls *a* and *d* from red algae showed that the recognizable contribution of chlorophyll *d* to the derivative spectrum is only a broad negative component, largest at 712 mμ in methanol, as shown in figure 5. The positive component of the chlorophyll-*d* derivative spectrum is completely masked by the large negative component of the chlorophyll-*a* curve. This appearance is consistent with the absorption spectrum recorded by the Beckman spectrophotometer for the same sample of mixed chlorophylls *a* and *d*.

Many red algae give little or no chlorophyll *d* on methanol extraction. Livingston and Holt have prepared derivatives of chlorophyll *a* having a spectrum identical to that of chlorophyll *d*. Smith, however, found Livingston's preparation to be separable from red algal chlorophyll *d* by chromatography. Holt has recently raised the question whether chlorophyll *d* actually exists in live algae or is a derivative of chlorophyll *a* formed in the extraction process. Though our work of limited scope on derivative spectra of a few red algae has not yet shown chlorophyll *d* *in vivo*, it can by no means substantiate the idea of its nonexistence *in vivo*. These experiments, however, have shown that the negative component peak at 700 mμ

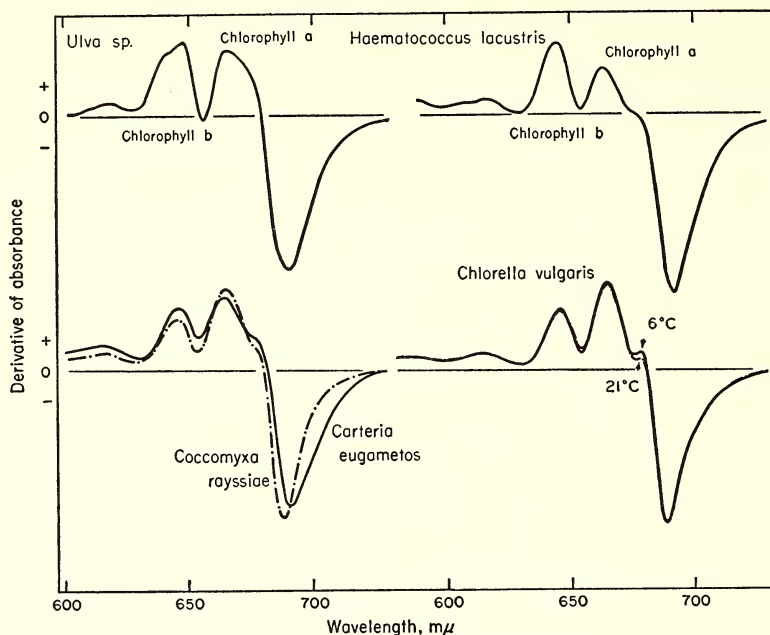


Fig. 4. Derivative spectra of algae containing chlorophylls *a* and *b*. We are indebted to Dr. Francis T. Haxo for the sample of *Ulva*.

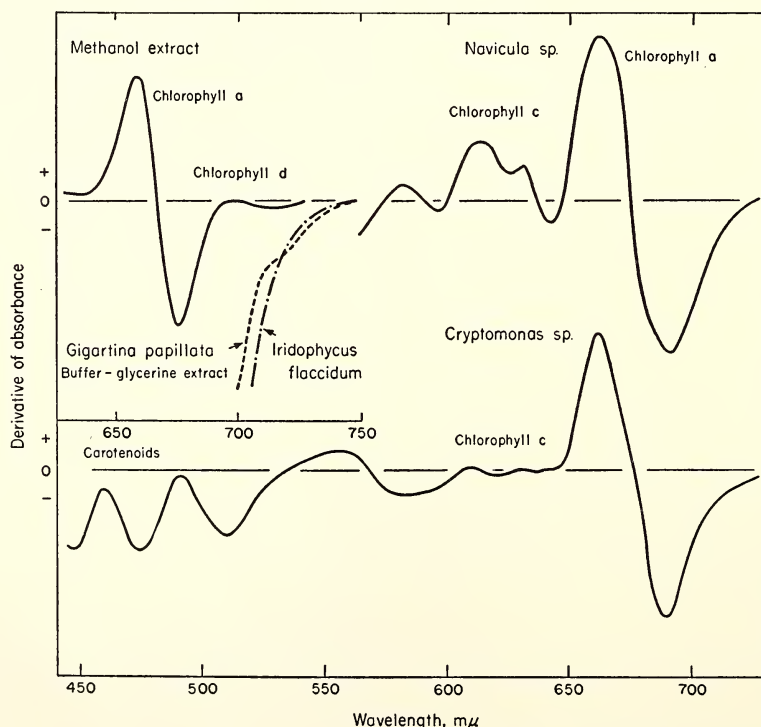


Fig. 5. Derivative spectra of algae containing chlorophylls *a* and *c* and a methanol extract of *Gloiophloea confusa* containing chlorophyll *d*.

in derivative spectra of old *Euglena*, discussed later, and of *Porphyridium aerugineum* is not chlorophyll *d*. Derivative spectra of organisms with chlorophyll *d* would show a broad negative component at about 720 m μ and would not have a

be useful in studying the shape of the red band of chlorophyll *a*. The absorption of phycocyanin may seriously overlap that of chlorophyll *a*, however, as can be seen in figure 6, illustrating spectra of algae with phycobilin pigments. In *Porphy-*

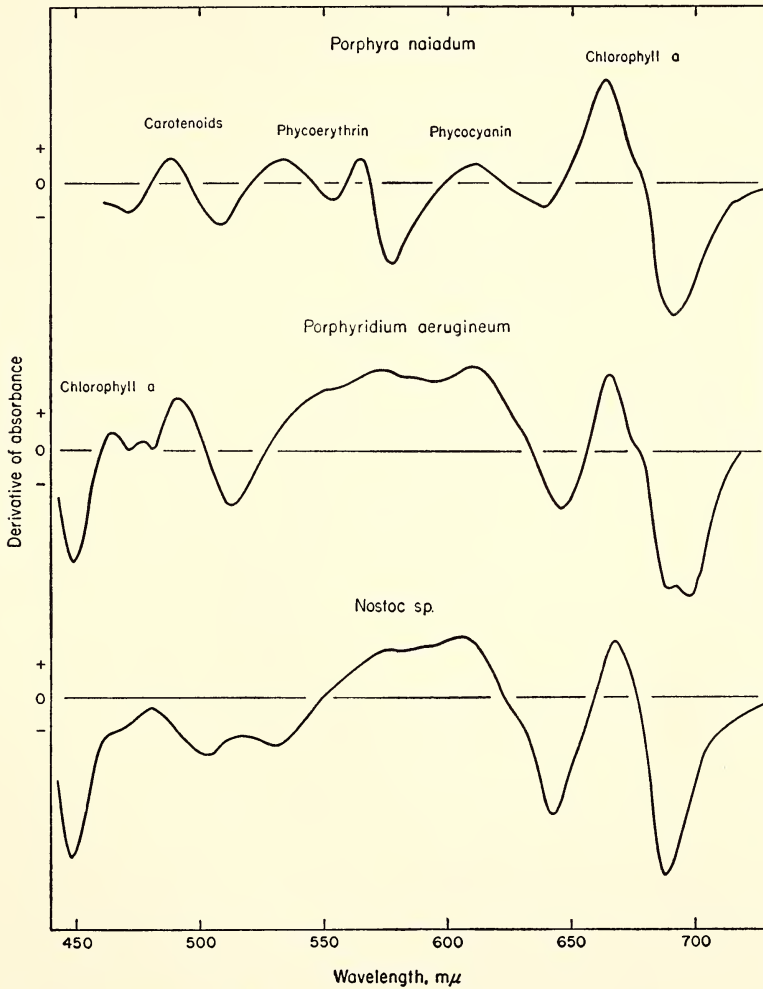


Fig. 6. Derivative spectra of algae containing chlorophyll *a*, phycoerythrin, and phycocyanin. We are indebted to Dr. Francis T. Haxo for *Porphyra* and *Porphyridium* and to Dr. J. A. Bassham for *Nostoc*.

recognizable positive component because of overlap by chlorophyll *a*.

Algae with phycobilin pigments. Since the blue-green and the red algae have only chlorophyll *a*, except for the presumed small and variable amounts of chlorophyll *d* in the reds, it was hoped that they might

ridium the negative derivative peak characteristic of old *Euglena* cultures appears at 700 m μ .

Curve analysis. Some attempts have been made to derive the curves of the individual chlorophyll-*a* components *in vivo* that appear to add together to give

the measured curves for the chlorophyll-*a* band of live algae. If we have curves for two algae, differing by only one component, it should be possible to derive the curve for that component by subtracting one algal curve from the other after appropriate adjustments of scale. Many difference curves so obtained were unsymmetrical and appeared to be made up of more than one component.

A chlorophyll-a band at 695 mμ in Euglena. One of the most striking observations made in this survey of algal absorption spectra was the sporadic appearance in *Euglena* of extra absorption at 695 mμ. It required an embarrassingly large number of experiments to find out that this band occurs in old, but not in young, cultures. Light intensity, temperature, media, and various species of *Euglena* were stud-

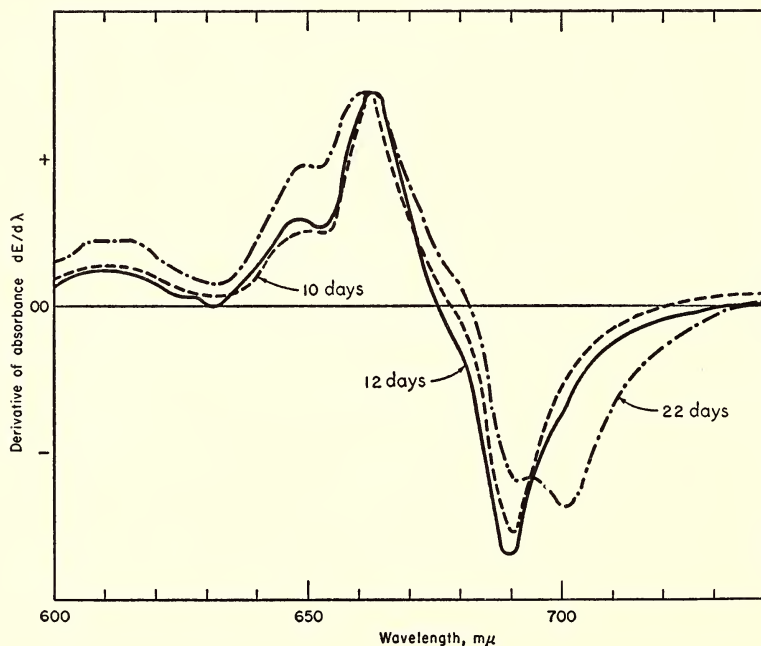


Fig. 7. Derivative absorption spectra of *Euglena* cultures of various ages.

Rather than elaborate the detailed analysis of complex curves we have continued the survey of different algae in the hope of finding pairs of curves more amenable to analysis by simple subtraction than those now available. Another approach to the derivation of the component curves lies in the possibility of selectively bleaching or interconverting the forms of chlorophyll *a* as described in another section of the report. A quantitative accounting for the shape of the whole curves for the algae in terms of the summation of curves representing individual components cannot yet be given.

ied. Any conditions that allow growth eventually produce the extra absorption band, and no conditions were found to do so immediately. We do not yet know what specific difference between young and old cultures, such as exhaustion of medium, accumulation of excretion products, or alteration of metabolism, may be the cause of the effect. In derivative spectra the extra absorption band shows as a sharp negative peak at about 700 mμ. The positive branch of the derivative curve of this component is obscured by the other chlorophyll-*a* forms present.

Figure 7 shows derivative spectra of

three cultures of *Euglena* grown to different ages. The progressive increase of the 700-m μ negative peak is apparent. Does this extra peak necessarily indicate the formation of a new pigment, or could it be an artifact due to some optical property of the cells? The density of cells used in the suspension measured does not materially influence the shape of the curve.

A reasonably sound argument in favor of the actual presence of an extra pigment is provided by the fluorescence spectra, figure 8, of the same cultures whose derivative spectra are presented in figure 7.

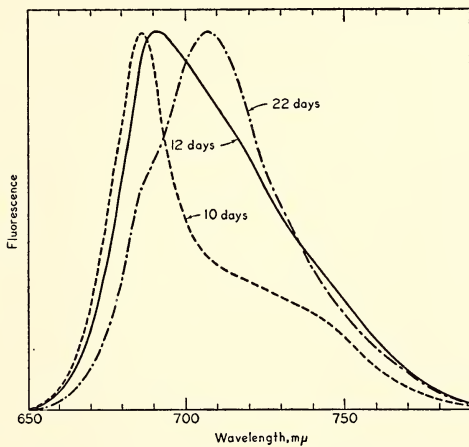


Fig. 8. Fluorescence spectra of *Euglena* cultures of different ages. The same cultures were used as in figure 7.

As the culture ages, the fluorescence at 710 m μ increases relative to that of "ordinary" chlorophyll *a* *in vivo* at about 685 m μ . The curves are not corrected for the wavelength variation of the sensitivity of the photomultiplier (Du Mont #6911). The progressive accumulation of a component fluorescing at 710 m μ is shown. The suspensions used were thin enough to avoid distortion of the fluorescence through reabsorption by other cells. If these curves are distorted by reabsorption of fluorescence it must be by reabsorption within the individual cells.

To see how the spectra of young and old *Euglena* cultures look when plotted,

not as derivative spectra, but as absorbance itself against wavelength, two integrated curves are shown in figure 9 with scale adjustment to make them the same height.

The composite chlorophyll-*a* band in the old cultures is broadened on the long- but not on the short-wavelength side. The symmetrical flattening effect described by Duysens therefore cannot be the cause of the broadening. The selective scattering described by Latimer comes to mind. But all these algal spectra were measured with an opal-glass arrangement collecting a large part of the transmitted light, and furthermore a suspension of young cells does not show the effect, no matter how dense it may be.

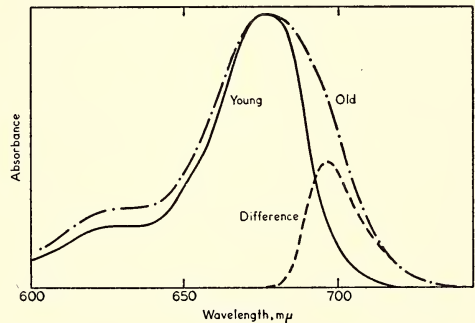


Fig. 9. Absorption spectra of a 9-day and a 36-day *Euglena* culture adjusted to the same height. The curves were integrated from the measured derivative spectra.

To see whether the extra absorption at long wavelengths could be attributed to greater scattering in larger pigment particles, old *Euglena* cells were disintegrated by extrusion through a needle valve or by supersonic treatment. The whole homogenates, and centrifugal fractions of them, were examined. The 700-m μ negative derivative peak was appreciably smaller in the disintegrated than in the intact algae. This peak was, however, the same size in the different centrifugal fractions and in the whole homogenate. Even the supernatant after centrifugation at 10,000*g* for 30 minutes gave the same spectrum as the entire homogenate, indicating that the ex-

tra peak is found not only in large particles.

Several batches of old *Euglena* were extracted with acetone, and the spectra of the extracts were measured directly or after transfer to ether. No other chlorophyllous pigments than *a* and *b* were present. No extra bands showed on chromatograms made by Dr. Smith. The extra pigment of old *Euglena* is therefore believed to be a form of chlorophyll *a in vivo* having an absorption peak at 695 m μ and a fluorescence peak at about 710 m μ .

Bleaching of Chloroplast Preparations by Red Light

J. S. Brown and C. S. French

The spectra of chlorophyll in live algae discussed elsewhere in this report appear to indicate the simultaneous presence of several forms of chlorophyll *a in vivo*. We hope eventually to fit the spectra of intact plants by adding together appropriate proportions of separate curves characteristic of the individual components. The experiments discussed here were undertaken to obtain the curves for the individual components by a more direct method than by curve analysis. The hope is to alter selectively the proportions of the components by some chemical or physical treatment. The curve for the component thus changed would be the difference in the spectrum before and after treatment.

There are strong indications in recent work of other laboratories that such a simple treatment as partial bleaching by light may selectively alter a single component contributing to the spectrum of a suspension of disintegrated chloroplasts. The Krasnovsky group has been able to bleach chloroplast material prepared in aqueous extracts from young sugar beet leaves to the extent of 20 to 30 per cent by irradiation with strong red light for 5 min. This bleaching is also reported to shift the absorption peak a few millimicrons toward longer wavelengths, an effect attributed to a selective destruction of the 670-m μ form

of chlorophyll *a*, leaving the longer-wavelength form undisturbed. We repeated these experiments with material from young Swiss chard leaves and found a bleaching effect but no shift in the absorption peak.

Methods. The leaves were harvested from the greenhouse in full daylight. All subsequent operations on the leaves were carried out in a darkened room. The large center vein was removed. The leaves were then ground in a mortar with about 50 ml of 0.1 *M* phosphate buffer at pH 7 and 0.02 *M* KCl. After being strained through a linen cloth to remove the larger debris, the mixture was centrifuged at 3500*g* for 10 min. The supernatant was diluted with more 0.1 *M* buffer to a convenient optical density for determining the spectrum in the derivative spectrophotometer. The Russian workers diluted their chlorophyll preparations with glycerine before measuring the absorption spectrum in order to "stabilize" the solution. We observed, as had the Russians, that the glycerine had no effect upon the absorption peak or the bleaching reaction. Since our material did not settle during the measurement, glycerine was not used. For bleaching, the solution in the cuvette was irradiated for 5 min in the focused beam of a tungsten lamp, the light passing first through water and a red Corning 2418 glass filter. The light intensity incident on the filter was about 100,000 fc.

Results. In our experiments the absorption curve in the chlorophyll-*a* region was not changed in shape but only diminished in height, as shown in figure 10. Chlorophyll *b* was more resistant to bleaching than chlorophyll *a*. We measured the derivative absorption spectrum in order to detect small changes in the shape of the absorbance curve. Our method would have readily detected any selective bleaching of a single component. A similar bleaching of preparations from Swiss chard was observed in phosphate buffers, at either pH 7.3 or 8.5,

used for the preparation and dilution of the sample.

The derivative absorption spectrum does not change if the samples, either before or after bleaching, are allowed to stand for an hour in the dark either at room temperature or in the cold room. Bleaching occurred when the temperature of the sample during measurement and irradiation with red light was kept at about 10° C as well as at room temperature (22° to 27° C).

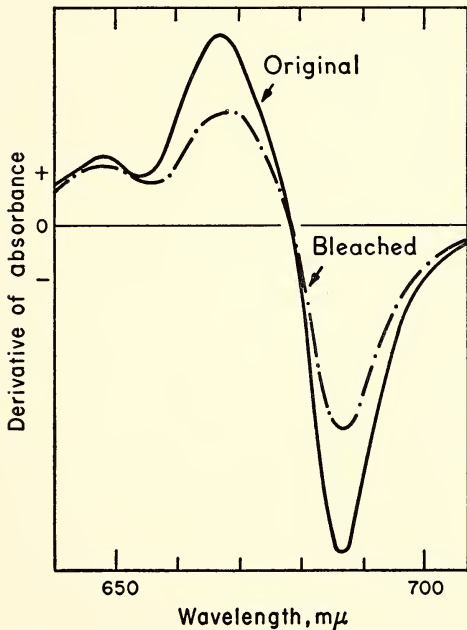


Fig. 10. Derivative spectra of Swiss chard chloroplast fragments before (solid line) and after (dot-dash line) bleaching.

Pokeweed leaves from plants just starting to blossom, and mature poplar leaves, were treated similarly to those of Swiss chard. No bleaching was observed with either.

The Krasnovsky group reports great variability in the susceptibility of preparations from leaves different in source, age, and environment. Our experiments are being continued, therefore, in the hope of finding suitable material to give selective bleaching of one chlorophyll component.

Chlorophyll Synthesis in Vivo and in Vitro

James H. C. Smith

Improvement of preparative methods for protochlorophyll holochrome. Many variations in the methods for the preparation and purification of the protochlorophyll holochrome have been tested within the year in the hope that a greater yield of a purer product might be obtained. Two beneficial variations have been found: one, to extract the etiolated bean leaves with the customarily used glycine buffer but with 20 per cent glycerol added; the other, to add barium chloride at the proper stage of purification to precipitate bothersome impurities. A summary of the modified method follows.

Approximately 50 g of etiolated bean leaves, harvested about 10 to 13 days after planting, were divided into 10-g lots, each of which was ground in a mortar with sand and 30 ml of 0.1 M glycine buffer, pH about 9.5, containing 20 per cent by volume of glycerol. All operations were carried out in a cold room with very weak green light. The combined extracts were squeezed through a linen cloth, and the filtrate was centrifuged for 15 min at 10,000g. The ice-cold supernatant solution was brought to 25 per cent saturation with ammonium sulfate and then to 45 per cent saturation, and was centrifuged at each concentration. The precipitate last obtained was taken up in 100 ml of 0.1 M glycine buffer (pH 9.5) and dialyzed against 2 liters of distilled water overnight. After dialysis, the solution was treated with 5 ml of barium chloride solution (40 per cent), and the precipitate was removed by centrifugation. The supernatant solution was brought successively to 25 then 40 per cent saturation with ammonium sulfate and was centrifuged after each treatment. The final precipitate was dissolved in 20 ml of glycine buffer. This stock solution, kept dark in the cold room, was used for quantum-yield measurements. Just before each measure-

ment it was centrifuged to remove whatever cloudiness had formed during storage. The solutions obtained by this procedure scattered light very little.

Various substances were added to the 0.1 *M* glycine buffer extractant in an attempt to obtain a larger quantity of more stable holochrome. Some of the substances added and the effects they produced are worth mentioning:

Urethane (20 per cent) did not affect the extraction of the holochrome but completely stopped its phototransformation. Digitonin (0.7 per cent) gave excellent extraction of the holochrome. It did not affect its phototransformation immediately after extraction, but it almost completely stopped the conversion after a 48-hr storage of the extract at 5° C. Sodium azide, potassium cyanide, ascorbic acid, 10 per cent methanol, and 10 per cent ethanol all gave good extraction and transformation of the protochlorophyll holochrome immediately after extraction, but they impaired either the stability or the transformability of the pigment when the extract was stored. Noteworthy was the fact that extracts containing ethanol developed the odor of acetaldehyde during storage. Acetaldehyde (5 per cent) gave good extraction and permitted good transformation of the pigment but gave rise to colored compounds during storage which obscured the absorption spectrum changes produced by illumination. The results, however, indicated that the transformation was comparatively good. Glycerol (20 per cent) gave an excellent extract of relatively good keeping qualities.

Because the glycine buffer containing glycerol gave a good extract in respect to quantity, stability, and transformability of the protochlorophyll holochrome, a solution composed of 0.1 *M* glycine + 0.05 *M* potassium hydroxide + 20 per cent glycerol is now being used in our laboratory routinely for extraction.

Several additives have been tried for improving the glycerol-containing extract, but

none of them has been of special value; some were harmful. In general, the presence of glycerol reduced the injurious effects of harmful additives. For example, digitonin almost completely stopped the conversion of protochlorophyll after storage in the glycerol-free medium, whereas it only partly inhibited its transformation in the glycerol-containing medium.

Other glucosidic additives, the saponins from *Quillaia*, *Saponaria*, and *Gypsophilla*, kindly supplied by Professor C. R. Noller, of Stanford University, were without significant effect either on the quantity of pigment extracted or on the quality of the extract. Neither *p*-chloromercuribenzoate alone nor in combination with digitonin was beneficial. The combination of the two actually promoted photodestruction of the new-formed chlorophyll. Iodoacetate had no influence on the extraction or the conversion of protochlorophyll immediately after extraction. Its effects on the stored pigment were not clear-cut and need further study. Sodium hydrosulfite was injurious to the extraction, conversion, and storage of the pigment.

Heretofore, the protochlorophyll holochrome was isolated from the crude extract and subsequently purified mainly by fractional precipitation with ammonium sulfate. Occasionally, other procedures of purification combined with ammonium sulfate fractionation have been tested, either to separate the holochrome directly or to remove troublesome impurities before isolating the holochrome. Freezing and thawing the extract was tried in order to precipitate impurities, but without success. Contrary to its behavior in leaves, the isolated holochrome was fairly resistant to this treatment. Neither purification by precipitation with lead acetate followed by dissolving the precipitate in Versene, nor adsorption on aluminum oxide, nor precipitation by protamine sulfate proved to be more useful for purification than ammonium sulfate fractionation alone. Among the innovations tried, however,

one was found to be highly advantageous, namely, dialysis of the partly fractionated material followed by treatment with barium chloride. After this treatment had been found to be successful in removing bothersome contaminants, it was routinely included in the preparative procedure.

Quantitative measurements of the protochlorophyll-chlorophyll transformation in the isolated holochrome have been somewhat hampered by the photodestruction of the new-formed chlorophyll. For example, when a given solution of holochrome was illuminated with a light intensity of about 400 fc from an electric lamp, the absorbances of the chlorophyll maximum at 1.0, 1.5, and 2.0 min of illumination were 0.625, 0.563, and 0.500—a decrease of 20 per cent during the 1 min of illumination. Conditions were sought for preventing this destruction. The presence of *p*-chloromercuribenzoate in the extract retards but does not prevent bleaching. Addition of digitonin or cysteine to such an extract increases its sensitivity to light. Iodoacetate in the extract tends to stabilize the solution somewhat. Sodium hydrosulfite does not prevent bleaching either when the solution is open to the air or when enclosed in a stoppered cuvette. (Sodium hydrosulfite added to the solution after transformation of the pigment lowers the absorption over the whole visible range, but chiefly at the shorter wavelengths.) Evacuation of the absorption tube is not entirely effective in stopping bleaching. As yet, no completely satisfactory conditions have been found for stopping the photodestruction of the new-formed chlorophyll.

Molecular properties of protochlorophyll holochrome. Previously it was reported that protochlorophyll holochrome has a particle weight of about 400,000, a value obtained from centrifugation measurements. The molecular weight has been newly estimated from the weight ratio of protochlorophyll to protein, assuming a molecular ratio of unity. These analyses have been made on lyophilized (freeze-

dried) material and also on solutions of the fractionated holochrome. The protochlorophyll was determined spectroscopically. The protein was estimated either from the weight of the lyophilized material directly, or from solutions of the holochrome by trichloroacetic acid precipitation, or colorimetrically with the biuret reaction. The molecular weights found ranged from 1.2×10^6 to 2.1×10^6 . The higher molecular weight obtained by analysis as compared with centrifugation can be attributed to contamination of the holochrome by foreign proteins, which may resemble the holochrome so closely that their separation by any means may be difficult or impossible.

Carotenoid pigment appears always to accompany the protochlorophyll holochrome derived from normal etiolated leaves. Whether this pigment is attached to a protein that accompanies the protochlorophyll holochrome as an impurity or whether it is an integral part of the holochrome remains uncertain. That it may be a constituent of the holochrome itself is suggested by the fact that the carotenoid is present in a molecular ratio of about unity to the chlorophyll formed during the protochlorophyll-chlorophyll transformation. In contrast to this, the carotenoid-protochlorophyll ratio is about one-half. From these figures it is evident that only about half the protochlorophyll present is transformed to chlorophyll and that what is transformed is statistically related in a molecule-for-molecule ratio to the carotenoid present.

It is not possible, however, to equate carotenoid content with degree of transformation, because in albino leaves nearly complete conversion occurs in the absence of carotenoids. It is more reasonable to assume that the yellow carotenoids act as an indicator for the presence of another substance accompanying the carotenoids in constant proportion which acts as the hydrogen donor in this photochemical reaction.

The carotenoid pigment has not yet been identified. In the distribution of the pigment between 80 per cent acetone and petroleum ether the pigment goes into the petroleum ether. This indicates that the yellow pigment is likely to be either a carotene or a xanthophyll ester rather than a free xanthophyll.

Etiolated leaves contain a mixture of phytylated and unphytylated protochlorophylls which is transformed to a corresponding mixture of chlorophylls by illumination. Subsequent to the transformation the unesterified pigment is almost completely changed into the esterified form. In the purified holochrome, however, only the unesterified form of the pigment exists, and this is not esterified by standing at room temperature after the illumination. By purification of the holochrome, all chlorophyllase activity has been eliminated. Whether this loss of chlorophyllase activity is caused by separation of the holochrome from chlorophyllase or from phytol, or from both, is not known.

The density of the acetone-extracted holochrome is 1.35. This value agrees very well with the values found for other proteins.

Chlorophyll mutants of corn: seed color and leaf pigment properties. This year the relation of seed color and pigment properties of etiolated leaves has been examined for three mutant strains of corn. Yellow and white seeds from each of three strains were used. Leaves from the dark-grown plants, before and after being illuminated, were examined spectroscopically with the opal-glass spectroscopy accessory described in last year's report by Smith and Hart.

A notable correlation exists between seed color and yellow pigment in the leaves. The ratio of yellow pigment to protochlorophyll, measured as the ratio of absorbances at 480 and 647 m μ , was invariably less for leaves from white seeds than from yellow seeds of the same strain, namely, 1.3 vs. 18.2, 6.3 vs. 9.9, and 9.9 vs. 12.7.

Another correlation exists between seed color and chlorophyll accumulation. When leaves from yellow seeds were illuminated for 30 min following the initial protochlorophyll-chlorophyll transformation they accumulated chlorophyll, whereas leaves from white seeds did not. In two strains, the leaves from white seeds even lost a large proportion of the chlorophyll obtained by the initial conversion.

When briefly illuminated normal leaves are stored in the dark, the absorption band of the new-formed chlorophyll shifts in position from about 684 to 670 m μ . In the corn mutants under consideration, the leaves from all the yellow seeds and from two strains of white seeds showed the usual shift. Leaves from one white-seeded strain, however, showed only a slight shift, and its rate was very slow.

In respect to other properties—the degree of transformation of protochlorophyll to chlorophyll, and the percentage of unesterified protochlorophyll present in the dark-grown leaves—no difference was observed between yellow- and white-seeded strains.

The author is indebted to Dr. D. R. Robertson, of Iowa State College, for the seeds used.

Quantum Yield for the Protochlorophyll-Chlorophyll Conversion

J. H. C. Smith and C. S. French

For some years the mechanism of chlorophyll formation in higher plants has been under investigation in the Department. The study of the photochemical transformation of protochlorophyll to chlorophyll *a*, in which two hydrogen atoms are transferred to protochlorophyll from an unknown hydrogen donor, has been our special interest.

Previously it has been established (Year Book 47, p. 94, and Year Book 48, pp. 91-92) that this process is a molecule-for-molecule conversion for which the relative effectiveness of different wavelengths of light corresponds well with the absorption spectrum of protochlorophyll. These facts

left little doubt that protochlorophyll is the immediate precursor of chlorophyll *a* in the chlorophyll-forming process and that it is the active light-absorbing agent for its own conversion.

A further approach toward understanding the mechanism of the reaction has been made this year by determining the number of molecules transformed by each quantum of light absorbed by active protochlorophyll. This ratio, commonly called the quantum yield or quantum efficiency, was found to be about 0.6.

Heretofore, quantum-efficiency measurements of this process were not practical because of the high light-scattering and uncertain absorbancy properties of the systems available for measurement, such as etiolated leaves or cloudy extracts of the holochrome. The present measurements have been made possible because of the clear concentrated extracts of the active protochlorophyll holochrome that have recently been obtained.

Even with clear and concentrated solutions of protochlorophyll holochrome, there have been some complications, due to the nature of the system, in the measurement of quantum yields. These complications are that some inactive protochlorophyll is present which absorbs light but does not use it, and that the chlorophyll formed absorbs a progressively increasing fraction of the incident light whereas the decreasing active protochlorophyll absorbs a progressively decreasing fraction of the incident radiation. Since the method for determining the quantum yield was in some respects unconventional because of the complications just mentioned, the procedure followed in one experiment will be outlined.

Apparatus. Energy measurements were made with a Kipp and Zonen compensated thermopile-galvanometer system giving a deflection of 23.3 ergs/cm²/sec/mm. This system was calibrated with an incandescent electric lamp standardized by the National Bureau of Standards. Absorption

spectra were measured with a Beckman DK-2 recording spectrophotometer. The actinic radiation was of wavelength either 642 or 644 mμ. The first beam was isolated from a continuous source with a monochromator plus Corning filter 2412. Its half-width was about 5 mμ. The second beam came from a cadmium arc and was monochromatized by Corning filter 2412 + Chance 0N20 + 3 cm water.

Experimental procedure. The holochrome solution, prepared as described on page 287, was placed in a 1.00-cm absorption cell and exposed successively for 2, 4,

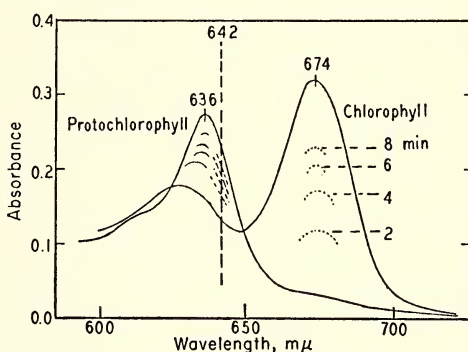


Fig. 11. Absorption measurements on the holochrome solution used for quantum-yield determinations of the protochlorophyll-chlorophyll conversion. At 674 mμ the absorbance due to chlorophyll increases with time of illumination, whereas at 636 mμ the absorption due to protochlorophyll decreases correspondingly.

6, and 8 min to light of wavelength 642 mμ, and an intensity of 6.64×10^{18} quanta/cm²/sec over an area of 2.63 cm². Subsequently, the solution was irradiated for 1 min with light from an incandescent electric lamp to convert the active protochlorophyll completely to chlorophyll. The absorption spectrum was measured before and after each successive illumination. (Cf. fig. 11.) Finally, the holochrome solution was diluted with enough acetone and water to bring it to 15.0 ml of 80 per cent acetone. The protein, which precipitated, was sedimented, and the absorption spectrum of the supernatant was measured. From Mackinney's value, 84 l/g cm for

the specific absorption coefficient of chlorophyll *a* in 80 per cent acetone, the number of molecules of chlorophyll formed and, correlatively, the number of molecules of protochlorophyll transformed were determined. By conversion of the absorbancy of chlorophyll in acetone to its absorbancy in holochrome solution, the number of pigment molecules converted during each irradiation period was calculated.

Usually, the quantum yield is determined by dividing the total number of molecules transformed by the corresponding total number of quanta absorbed by the reactant. In the present experiments, this procedure was beset with difficulties, which have already been enumerated. To avoid them, the initial rates of protochlorophyll transformation and of quantum absorption were used instead of the total quantities.

Initial rate of transformation. Previously, the phototransformation of protochlorophyll in live leaves was shown to follow strictly the second-order rate law, which has now been demonstrated to hold for holochrome extracts as well. By substitution of the data for the chlorophyll formed (cf. fig. 11) into the second-order equation for equal concentrations of reactants, a value for the initial concentration of protochlorophyll was obtained. The rate constant was also found from the classical equation. By substituting the values for the rate constant and the initial concentration of protochlorophyll into the derivative form of the second-order equation, $-(dc/dt)_{t=0} = kc^2$, the initial rate of protochlorophyll transformation was obtained.

Initial rate of quantum absorption. The initial absorption by the active protochlorophyll was determined as follows: The absorbance of the holochrome solution at 642 m μ was measured before exposure to light and after complete conversion had occurred. (Cf. fig. 11.) The difference between these values was the algebraic sum of the decrease in absorbance by the active protochlorophyll and the increase in ab-

sorbance by the chlorophyll. This difference plus the estimated increase in chlorophyll absorbance gave the true initial absorbance by the active protochlorophyll. The increase in chlorophyll absorbance was estimated from the known concentration of chlorophyll and its specific absorption coefficients in organic solvents after proportional adjustments had been made for wavelength shift and absorbance ratios between the holochromatic and organic solutions of the chlorophyll.

The fraction of the incident light initially absorbed by the solution was calculated from the initial absorbance of the solution at 642 m μ . This absorption multiplied by the ratio of the active protochlorophyll absorbance to total absorbance gave the fraction of the incident light initially absorbed by the active protochlorophyll. This value multiplied by the quantum flux gave the initial rate of absorption of quanta.

Quantum efficiency. The quantum efficiency was then found by dividing the number of molecules initially transformed per second by the number of quanta initially absorbed per second by the active protochlorophyll. In this experiment the value found was 0.63 molecule/quantum.

Other procedures. The procedure just described was varied in other experiments. Another reaction vessel was used, which presented a larger area (5.80 cm²) and a thinner layer (0.241 cm) of holochrome solution to the incident beam. Instead of submitting the same solution to several successive exposures, equal volumes of the same stock solution were used for successive exposures, and the pigment was transferred to 80 per cent acetone after each exposure for determining the number of molecules transformed. Different monochromatic beams were tried: one the filtered beam from a monochromator (642 m μ), another the filtered spectral line from a cadmium arc source (644 m μ). In one instance the absorption of the actinic beam by the reacting solution was meas-

ured directly. Even with these variations, the values obtained for the quantum yield were in substantial agreement. The four values found were 0.63, 0.48, 0.70, and 0.61, the average being 0.60.

Discussion. Before the value 0.6 is accepted as final for the quantum yield of this process, it should be measured under a greater number of conditions and with more refined procedures. It is unlikely, however, that the value will be greatly changed. The value already obtained demonstrates that the conversion is an efficient photochemical process.

The chemistry of the conversion requires that two hydrogen atoms be transferred to protochlorophyll. Since the quantum yield is close to 0.5, and might ultimately be found to have this value, it is tempting to speculate that each quantum transfers a hydrogen atom.

The Polarization of Fluorescence from the Protochlorophyll Holochrome

Paul Latimer and James H. C. Smith

Recent studies of the protochlorophyll holochrome indicate that it is composed of a globular protein, M.W. 400,000, to which one or more protochlorophyll molecules are attached. Analyses of the purest preparations yet obtained indicate that on the average one or two protochlorophyll molecules are attached to each protein molecule. Improved techniques for purifying the holochrome would give a more reliable ratio of protochlorophyll to protein, but they could not give specific information about the distribution of the protochlorophyll molecules on the protein molecules; i.e., they would fail to distinguish between the following possibilities: (1) the same number of protochlorophyll molecules being attached to each protein molecule, and (2) many protochlorophyll molecules being attached to certain protein molecules while most of the protein molecules are free of protochlorophyll. The experiments described here were carried

out in an attempt to distinguish between these two possibilities.

It is generally known that fluorescence excited by polarized light may under some circumstances itself be partly polarized. The polarization of the fluorescence (which may be thought of as a memory of the polarization of the exciting light) depends on several factors, including the wavelength of the exciting light, the lifetime of excitation, the viscosity of the medium, and the size of the excited molecule (together with anything to which it may be attached).

Through studies of the polarization of fluorescence from the various chlorophylls, Goedheer and others have obtained valuable information about the modes of excitation of these compounds. Through somewhat similar studies of the fluorescence of chlorophyll in the green alga *Chlorella*, Arnold and Meek obtained direct experimental evidence of the existence of energy transfer between chlorophyll molecules *in vivo*. They showed that the fluorescence is almost completely depolarized and that the depolarization must have been brought about by energy transfer.

We measured the polarization of fluorescence from the chlorophyll holochrome in an effort to obtain similar evidence of energy transfer between chlorophyll molecules on a given protein molecule. Such evidence, if it could be obtained, would rule out the possibility that most of the holochrome molecules contained one and only one chlorophyll molecule. In these experiments, the chlorophyll holochrome was freshly formed from isolated protochlorophyll holochrome during excitation of the fluorescence.

The holochrome is known to be a spherical "particle" ~ 100 Å in diameter. According to Förster, excitation energy may be readily transferred between chlorophyll molecules across distances up to 80 Å. By assuming a random spatial distribution of two chlorophyll molecules on each protein molecule, we estimated that energy transfer

should diminish the polarization of fluorescence to 75 to 80 per cent of its "unreduced" value. A larger number of chlorophylls per protein would, of course, lead to a much greater depolarization. To determine the "unreduced" value of the polarization of fluorescence, we extracted the chlorophyll from the same light-exposed holochrome with acetone, dissolved the chlorophyll in castor oil, and measured the polarization of its fluorescence. Because castor oil is an exceedingly viscous solvent, there is very little depolarization of the fluorescence.

from either chlorophyll in acetone or fluorescein in water. Small corrections were applied to the experimental data to obtain the results presented in table 3.

The polarization values for chlorophyll *a* in castor oil and in *Chlorella* are in good enough agreement with those reported by other investigators to support the reliability of our experimental method.

It is seen that the fluorescence from the chlorophyll on the holochrome is polarized to approximately the same degree, whether it is attached to the protein or dissolved in castor oil (where the average distance be-

TABLE 3. Results of Fluorescence Polarization Measurements

Fluorescent Material	Medium	Wavelength of Exciting Light, mμ	Polarization $P = \frac{I_{II} - I_{I}}{I_{II} + I_{I}}$
Chlorophyll holochrome (partly converted)*	Buffer	405 436	0.12-0.14 0.05
Acetone extract of holochrome (includes both protochlorophyll and chlorophyll)	Castor oil	405	0.12
Chlorophyll <i>a</i> in viscous solvents	Castor oil	405 436	0.17 0.05
Chlorophyll <i>a</i> in <i>Chlorella</i>	Buffer	405	0.00-0.02

* Whereas light will convert nearly all the protochlorophyll to chlorophyll when the holochrome is in the leaf, only about 50 per cent conversion can be obtained with the isolated holochrome.

The fluorescence was excited with mercury lines isolated with interference and colored-glass filters. (We estimated that the exciting beams contained over 90 per cent light of the nominal wavelengths.) Fluorescence emitted at 90° to the incident beam was isolated with red filters, and measured with a photomultiplier tube. The polarizer, placed in the exciting beam, and the analyzer, in the fluorescent beam, were Polaroid plates. The fluorescent material was put in a specially constructed vessel ("Wood's Horn") to minimize detection of the exciting light. The relative sensitivity of the detecting system to light of different planes of polarization was determined by rotating a Polaroid disk concentrically with the detector in a completely depolarized light field. The source of such a light field was the fluorescence

tween chlorophyll molecules is far too great for energy transfer to occur). Hence, little if any depolarization could have been produced on the holochrome by energy transfer. If Förster's calculations are correct, the presence of just two chlorophylls on each protein should have led to a 20 to 25 per cent depolarization, which we could have detected. On this basis, the results indicate that most of the chlorophyll (or protochlorophyll) molecules must occur singly on protein molecules.

After our measurements were made we learned of unpublished calculations of R. Marcus which modify the above conclusions. According to Marcus, energy may be transferred between chlorophyll molecules only over a distance of 29 Å (instead of 80 Å as reported by Förster). Now, 20 to 25 per cent depolarization could have

been produced by no less than five to ten chlorophylls on each protein instead of two, if the value 29 Å is correct. Unfortunately, we could not have detected depolarizations of less than 10 per cent. Hence, we can only conclude from the above experiments that chlorophyll molecules do not occur in large numbers on single protein molecules of the protochlorophyll holochrome.

It appears that refinements in the experimental apparatus and checks of Förster's and Marcus' calculations will be needed in further studies along these lines. Such investigations may provide a type of valuable information about protochlorophyll (and perhaps other systems) that cannot be obtained through any other known technique.

Reversible and Irreversible Changes of Bacteriochlorophyll in Chromatophores

J. C. Goedheer

A comparison between properties of photosynthetic pigments when present in their natural state and when dissolved in organic solution is of importance for the understanding of their function *in vivo*.

Previous experiments on bacteriochlorophyll in organic solvents performed at the Biophysical Research Group at Utrecht have showed that this pigment can be bleached by the addition of ferric chloride, iodine, or potassium permanganate in various organic solvents. This oxidative bleaching results in a disappearance of the long-wavelength absorption band, located in most solvents around 770 mμ. The original spectrum can be restored by the addition of ferrous salts or ascorbic acid. For the oxidation-reduction potential of the system bacteriochlorophyll-oxybacteriochlorophyll a value of 300 mv versus saturated calomel electrode has been found. The bleaching of bacteriochlorophyll by light in the presence of air has also been studied.

The present investigations at the Department of Plant Biology are concerned

with the study of these properties of bacteriochlorophyll in its natural state. We therefore use chromatophores of photosynthetic bacteria. Crude suspensions of chromatophores were obtained by pressing the bacteria through a needle-valve homogenizer and removing whole cells and debris by low-speed centrifugation. Purified suspensions were obtained by sedimenting the chromatophores at high speed and suspending the sediment in buffer solution at pH 7.5. In most cases bleaching experiments were performed with purified as well as with crude suspensions. The experimental results follow.

Reversible oxidative bleaching in chromatophores of Rhodospirillum rubrum. With *Rhodospirillum rubrum* chromatophores the addition of ferric chloride resulted in a decrease of absorption of the long-wavelength band at 880 mμ. The addition of ferrous sulfate or ascorbic acid partly restored this band, as it did when bacteriochlorophyll was dissolved in organic solvents. The addition of ferric chloride decreased the pH of the solution considerably, with resultant disappearance of the weak absorption band at 800 mμ, which, unlike the main band at 880 mμ, had been found earlier to be sensitive to low pH.

The oxidation-reduction potentials of bacteriochlorophyll in the chromatophores were such as to allow ferri-ferrocyanide mixtures to be used as oxidation-reduction reagents in aqueous solutions. These mixtures could be employed in solutions buffered at pH 7.5. To produce the greatest bleaching effects potassium permanganate had to be used.

In figure 12 the results are given for the addition of ferri-ferrocyanide mixtures with different potentials and of potassium permanganate. The figure shows that two changes occur: the 880-mμ band is bleached, and the 800-mμ band is shifted in position from 805 to 799 mμ but is not bleached. Even at a potential producing a total bleaching of the 880-mμ band, the

800-m μ band is not bleached. The potential at which the 800-m μ band is shifted to its halfway position is approximately 520 mv against the hydrogen electrode whereas that at which the 880-m μ band is half bleached is approximately 650 mv. The bleaching of the 880-m μ band is not

used. If present, such a shift may have been masked by reversible bleaching. An irreversible shift toward shorter wavelengths was obtained when small quantities of iodine were added to an ether solution of bacteriochlorophyll. This resembles the shift of chlorophyll *a* on being

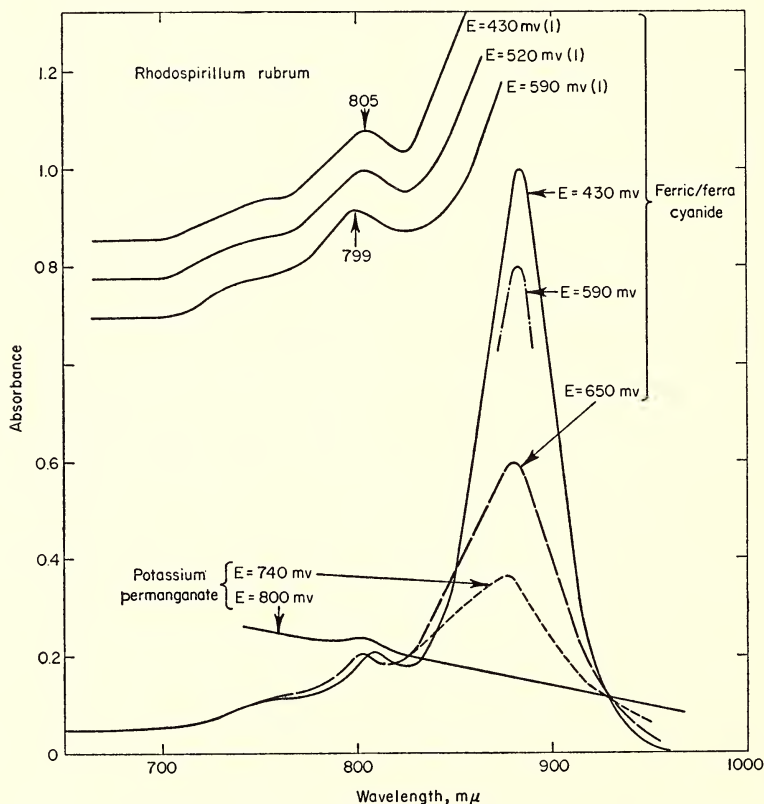


Fig. 12. Long-wavelength absorption spectrum of *Rhodospirillum rubrum* at different potentials. The values measured versus a saturated calomel electrode were converted to those based on the normal hydrogen electrode by addition of 250 mv. In the main figure the decrease in absorption of the 880-m μ band is demonstrated; the insert shows the shift in position of the 800-m μ band.

fully reversible: the sooner the reductant is added after the oxidation, the higher the percentage of reversibility. This behavior is similar to that in organic solution, where complete reversibility is obtained only when the reductant is added immediately after oxidation has taken place.

A reversible shift in position of the long-wavelength band was not encountered when solutions in organic solvents were

allomerized. The previously mentioned shift of the 800-m μ band in the chromatophores, however, may be caused not only by a change in pigment but also by a change in the protein part of the complex, assuming the bacteriochlorophyll bands *in vivo* to be affected by a pigment-protein linkage.

Similarity of change in absorption due to addition of oxidant and to illumination

of live *Rhodospirillum rubrum*. The differences between absorption spectra of suspensions of chromatophores from *R. rubrum* with and without addition of various ratios of ferri to ferrocyanide are shown in figure 13. The difference-spec-

allowed a determination of the exact location of the maximum of the 800-m μ band. Side illumination with blue light (isolated by a 5-cm CuSO_4 solution and a Corning 5032 filter) with an intensity of 1.1×10^4 ergs/cm 2 /sec produced a band shift from

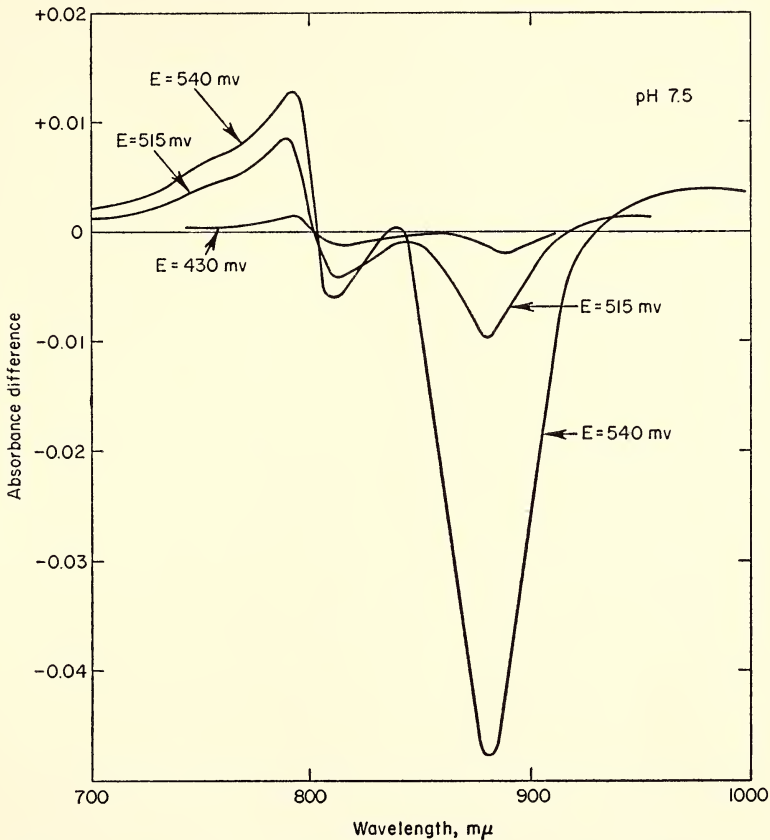


Fig. 13. Difference spectra between *Rhodospirillum rubrum* extracts treated with ferri-ferrocyanide mixtures and untreated extracts. The curve measured at 515 mv is similar in shape and in absolute magnitude to the difference spectrum between illuminated and nonilluminated intact bacteria measured by Duysens.

trum curve obtained at 515 mv is similar to that found by Duysens when he illuminated live bacteria suspended in distilled water (Year Book 52, pp. 157-160).

Not only are the shapes of the difference spectra similar, but also the absolute values of the changes in absorption are of the same order of magnitude. The positions of the absorption bands were measured in the derivative spectrophotometer, which

805 to 800 m μ . During storage in the dark for a few seconds this shift was reversed. When the intensity was reduced to a tenth, the shift was 3 m μ . The higher intensity, therefore, was sufficient to saturate the process.

The similarity of the difference spectra mentioned indicates that, under the conditions used, absorption of light *in vivo* results in an oxidation of bacteriochlorophyll

at a potential of 515 mv. In bacterial extracts the shift upon illumination was only 1 or 2 $m\mu$, whereas addition of ferricyanide to the same extract caused a shift of 5 $m\mu$.

Reversible oxidative bleaching of chromatophores of Rhodopseudomonas spheroides. Experiments also were performed with extracts of *Rhodopseudomonas spheroides*, which show a more complicated near-infrared absorption spectrum, namely, bands at 800 $m\mu$ and 850 $m\mu$ and a shoulder at 890 $m\mu$. Only the band corresponding to the 890- $m\mu$ absorption—the band to which according to Duysens all absorbed light energy is transferred—could be bleached reversibly by the addition of ferri-ferricyanide mixtures. At other wavelengths in the near-infrared spectrum a difference was encountered between old and fresh extracts. “Old” extracts were those kept for several days in the refrigerator; “fresh” extracts were those used immediately after their preparation. The difference spectrum for old extracts is similar to that of *Rhodospirillum rubrum* also treated with ferri-ferricyanide. Apparently here a small fraction of the 800- $m\mu$ band is shifted reversibly upon addition of the oxidant. The major part of the 800- $m\mu$ band and the 850- $m\mu$ band remains unchanged. Irreversible bleaching of these bands occurs only at potentials >700 mv. In fresh extracts irreversible bleaching of the 800- $m\mu$ band occurs at low potentials. Most probably also here a small fraction of this band is shifted reversibly to shorter wavelengths. This is shown in figure 14 as a maximum at about 795 $m\mu$ that disappears after addition of reductant. Irreversible increase in absorption arises simultaneously at about 680 $m\mu$. The irreversible decrease at 800 $m\mu$ and increase at 680 $m\mu$ are strongly enhanced at lower pH. The difference spectrum of a fresh extract buffered at pH 5.1 is given in figure 15.

Active chromatophores of *Rhodospirillum rubrum* and *Rhodopseudomonas spheroides* reduce ferricyanide in the dark upon addition of succinate. This phenome-

non, which occasionally could be accelerated by light, was used as a test for the reversibility of the bleaching effects. The addition of succinate caused disappearance of the difference spectrum of figure 14 and of the long-wavelength band in figure 15.

Bleaching of Rhodospirillum rubrum chromatophores at high light intensities. The influence of light on bacteriochlorophyll in its natural state under aerobic con-

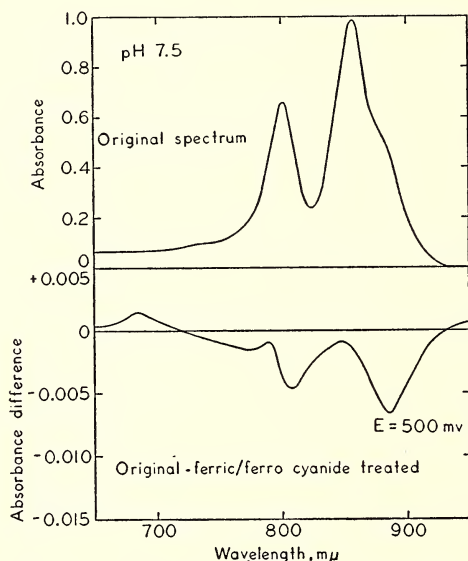


Fig. 14. Absorption spectrum and difference spectrum of a “fresh” extract of *Rhodopseudomonas spheroides* treated with ferri-ferricyanide with a potential of approximately 500 mv (pH 7.5). The band at 800 $m\mu$ in the lower spectrum is partly irreversible; the absorption increase at 680 $m\mu$ is irreversible.

ditions also was studied. The quantum efficiency of irreversible bleaching under illumination with red light was less than one-thousandth that for bacteriochlorophyll dissolved in methanol or acetone. Bleaching at high light intensities also proved to be dependent on the age of the extract: freshly prepared extracts were bleached 4 to 8 times more rapidly than extracts that had been kept for a week or longer in the refrigerator. The bleaching at high intensity was found to be partly reversible. A difference spectrum between a bleached

and an unbleached extract showed a decrease of the 880-m μ band and a shift of the 800-m μ band. A small increase found at about 855 m μ may point to a shift of a fraction of the 880-m μ band toward shorter wavelength. A partial reversibility of bacteriochlorophyll bleaching had been found also in organic solvents. The appreciable increase in the reversibility in methanol (but not in other organic solutions)

green oxidation product of bacteriochlorophyll having a chlorophyll-*a* type of spectrum. With old extracts the bleaching of the 800-m μ band was of the same order of magnitude as that of the other bands. This bleaching behavior at high light intensities resembles that with ferricyanide. The difference spectrum between a bleached and an unbleached solution (fig. 16) indicates that all three bands are

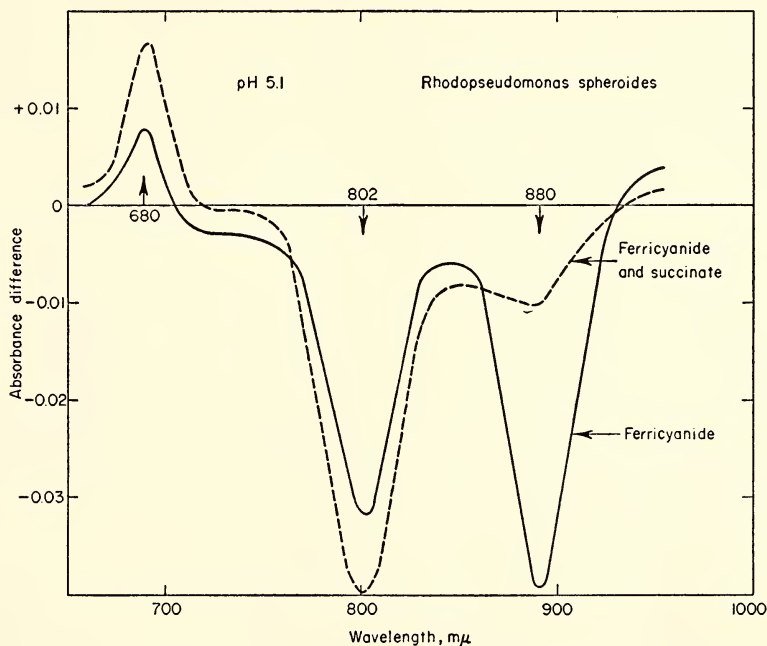


Fig. 15. Addition of succinate to the treated extract results in a reduction of ferricyanide. As the potential decreases the difference spectrum changes, showing the reversibility of the long-wavelength band and the irreversibility of the other bands. At this pH the irreversible bleaching is strongly increased.

obtained by addition of ascorbic acid did not occur with bacteriochlorophyll in the chromatophores.

Bleaching of *Rhodospseudomonas spheroides* chromatophores at high light intensities. In chromatophores from *Rhodospseudomonas spheroides*, bleaching by high light intensities also depended on the age of the extract. In fresh extracts the 800-m μ band was bleached appreciably faster than the other bands, and a simultaneous increase in absorption occurred near 680 m μ . The increase was found to be due to a

bleached and the bleaching is partly reversible. In this respect bleaching by light differs from the results with ferricyanide.

Influence of temperature on the spectra of bacterial chromatophores. Difference spectra of heated and unheated solutions of *Rhodospseudomonas spheroides* chromatophores proved to have a shape similar to the one in figure 16. The high light intensity causes an increase in temperature in the extract of about 3° to 5° C. It is possible, therefore, that this spectrum is at least partly due to heating. With *Rho-*

dospirillum rubrum it could be shown that the effect due to heating was only a small fraction of the total effect.

The effects may be due to a change in selective scattering caused by heating. The light-gathering angle of the Beckman DK-2 is small, and very little scattered light is received by the detector. Change of temperature results in change of refractive index and thus of scattering. Change in selective scattering in the neighborhood of sharp absorption bands might cause an apparent change in absorbance and position of an absorption band. In order to check this theory the refractive index of

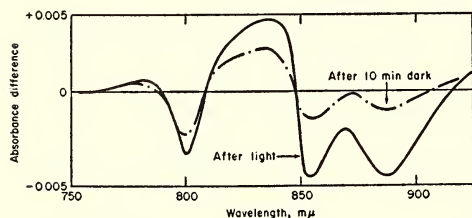


Fig. 16. Difference spectrum between an unilluminated extract of *Rhodospseudomonas spheroides* and one illuminated for 15 min (1000-watt lamp, Corning 3480 filter, and 5 cm water, intensity approximately 150,000 fc measured with the filters removed). This difference spectrum is similar in shape to the spectrum obtained after heating the extract.

the solvent was raised to 1.42 by addition of albumin. Such an addition would alter the absorption band if selective scattering had an influence. Although the over-all scattering of the extract increased (measured at 1000 mμ where no absorption occurs), no influence on the position of the maxima was noticed. Also the difference spectra were not influenced in the way to be expected if the effect were due to selective scattering. It thus seems unlikely that the changes measured upon heating are due to a change in scattering.

Difference spectra obtained by heating or cooling *Rhodospirillum rubrum* extracts showed a shift of the 800-mμ band toward longer wavelengths after cooling and toward shorter wavelengths after heating. These changes in the spectrum become ir-

reversible at relatively low temperatures. After 1 min of heating at 74° C the 880-mμ band of bacteriochlorophyll is decreased 80 per cent, and a new band arises at 780 mμ. One minute at 66° C decreases the 880-mμ band 25 per cent; 1 min at 56° C, 6 per cent. Results obtained with extracts of *Rhodospseudomonas spheroides* were about the same. The temperatures at which the absorption spectrum changes irreversibly thus are far below the boiling point of water, and of an order of magnitude similar to those found in green plants. No significant change occurs at these temperatures if bacteriochlorophyll is dissolved in organic solvents.

These experiments show both the similarities and the dissimilarities in the properties of bacteriochlorophyll in organic solvents and in chromatophores. The behavior of the *Rhodospseudomonas* bands, all of which correspond to a single bacteriochlorophyll band in organic solution, shows that the way this pigment is attached in the chromatophores is of great importance for its chemical (and probably photochemical) behavior. Whether the similarity of *in vitro* and *in vivo* difference spectra indicates that reversible oxidation of bacteriochlorophyll represents the primary reaction in bacterial photosynthesis or merely a side reaction cannot yet be decided.

Some Properties of Carotenoids in Bacterial Chromatophores

J. C. Goedheer

Lynch and French (Year Book 55, p. 250) demonstrated that when chloroplasts of green plants are treated with petroleum ether, which removes carotenoids and other compounds soluble in petroleum ether, their photochemical activity drops nearly to zero. Returning the petroleum ether extract to the dry chloroplasts and evaporating the solvent restores photochemical activity to the chloroplasts. The photochemical activity was measured by dye reduction. In chloroplasts the light

absorption by chlorophyll and carotenoids overlaps, and it is difficult to obtain a quantitative relation between carotenoid content and photochemical activity. In photosynthetic bacteria, however, the absorption bands of the carotenoids are located in a region of minimum absorption by bacteriochlorophyll, making these organisms suitable for a study of the properties of carotenoids *in vivo* or in isolated chromatophores. It seemed worth while, therefore, to repeat the experiments of Lynch and French with chromatophores from photosynthetic bacteria. Because the preparations of bacterial chromatophores did not reduce dye photochemically as green chloroplasts do, photophosphorylation by the chromatophores was used to test for the loss and restoration of photochemical activity.

Homogenates of purple bacteria were made by the needle-valve method. After intact bacteria and cell debris had been removed by centrifugation the supernatant was lyophilized immediately. Lyophilization rarely impaired the photophosphorylative activity by more than 30 per cent.

The photophosphorylation was induced by wavelengths longer than 580 m μ , which are absorbed by bacteriochlorophyll only. The intensity of the actinic light was approximately 800 fc (as measured with the Corning 3480 filter removed). To increase the phosphorylation, phenazine methosulfate, adenosinediphosphate, and succinate were added to the medium.

The uptake of inorganic phosphate was determined colorimetrically. In some cases an uptake of 550 μ M of phosphate per hour was obtained with extracts having an absorbance of 1.0 at 800 m μ . According to Frenkel's values, this absorbance corresponds to approximately 1 mg of protein per milliliter.

Extraction of carotenoids by petroleum ether (b.p. 60° C) proceeded much less readily with these chromatophores than with chloroplasts. Extraction of an appre-

ciable fraction of the carotenoids from *Rhodospirillum rubrum* chromatophores resulted only after prolonged treatment (20 to 30 min) with a homogenizer consisting of a plastic plunger in a test tube. After such treatment, the solubility of the chromatophore material was markedly decreased. The spectrum of the material before and after an extended carotenoid extraction is shown in figure 17. Absorption is appreciably decreased in the carotenoid region, but comparison with the spectrum of bacteriochlorophyll in organic solvents shows that some carotenoid is still left. It proved impossible to take all carotenoid out by the extraction method used. The percentage removed was estimated spectroscopically.

Photophosphorylation drops roughly in proportion to the amount of carotenoids removed, as is shown in table 4. After the

TABLE 4. Decrease of Photochemical Activity of Chromatophores of *Rhodospirillum rubrum* with Decreasing Carotenoid Content after Petroleum Ether Extraction

For each sample the ratios of carotenoid content and photophosphorylation are given with respect to their values in the untreated chromatophores.

Carotenoid Content	Photophosphorylation	Time of Extraction, min
1	1	0.5
0.95	1	1
0.90	0.87	5
0.35	0.22	30

petroleum ether solution containing the carotenoids (and possibly other dissolved compounds) was returned to the dry extracted material and the solvent evaporated, the material was almost totally insoluble, so that no measurement of a possible regeneration of photochemical activity could be made. This same treatment also lowered the capacity of the chromatophores to reduce ferricyanide in the dark in the presence of succinate. Whether the deactivation is caused by removal of carote-

noids or unknown compounds, or by a change in the structure of the chromatophores, remains uncertain.

Although the photochemical activity after addition of the extracts could not be measured, several observations of interest were made in the course of the experiments. Besides the carotenoid absorption changes, a small change in the bacteriochlorophyll absorption was found after

sulted in the partial irreversible bleaching of bacteriochlorophyll and the completely irreversible bleaching of the carotenoids. The proportion of the carotenoids bleached was equal to the proportion of the bacteriochlorophyll that was bleached irreversibly. Bleaching experiments were also performed with chromatophores from *Rhodospseudomonas spheroides*. In these chromatophores, bacteriochlorophyll has dis-

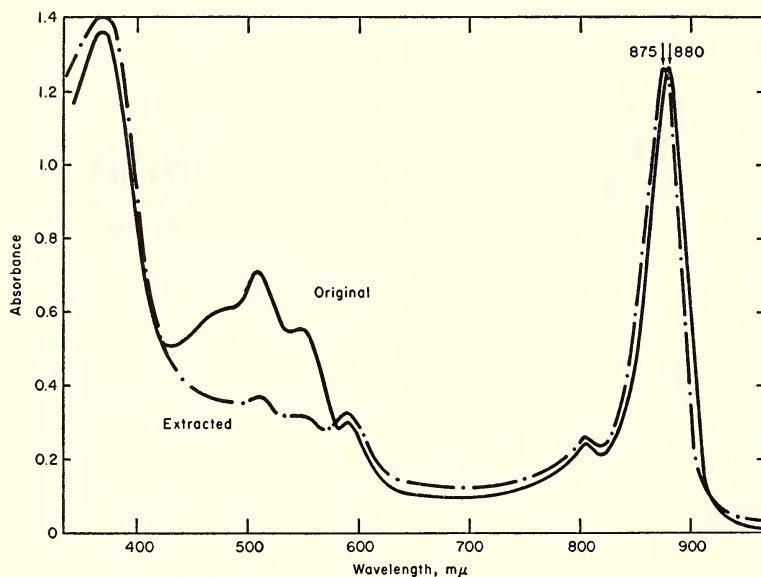


Fig. 17. Absorption spectrum of a water suspension of *Rhodospirillum rubrum* particles before and after extraction of carotenoids with petroleum ether. The absorption in the carotenoid region is markedly decreased after extraction, and the 880-m μ band of bacteriochlorophyll is shifted slightly toward shorter wavelengths.

extraction of the carotenoids. The 880-m μ band was shifted to 875 m μ as shown in figure 17. Possibly this shift is due to a change in the interaction of bacteriochlorophyll and carotenoid molecules.

Bleaching of bacteriochlorophyll at high light intensities results in a concomitant bleaching of the carotenoids, even by light that is not absorbed by them. The partial regeneration of bacteriochlorophyll after this photochemical bleaching does not restore the carotenoids. No significant reversal of the carotenoid absorption after illumination was observed.

Addition of ferric and ferrous ions re-

tinct absorption bands at 800 and 850 m μ and an absorption shoulder at 890 m μ . The experiments showed that under one set of conditions part of the carotenoids were bleached during the irreversible bleaching of the 800-m μ absorption band. Under other conditions only the 890-m μ shoulder was bleached and part of the carotenoids were bleached concomitantly. Furthermore, if such conditions were used in each of the experiments just mentioned that the 850-m μ band was also bleached, the carotenoid absorption completely disappeared. Thus it seems that a fraction of the carotenoid bleaching is associated with

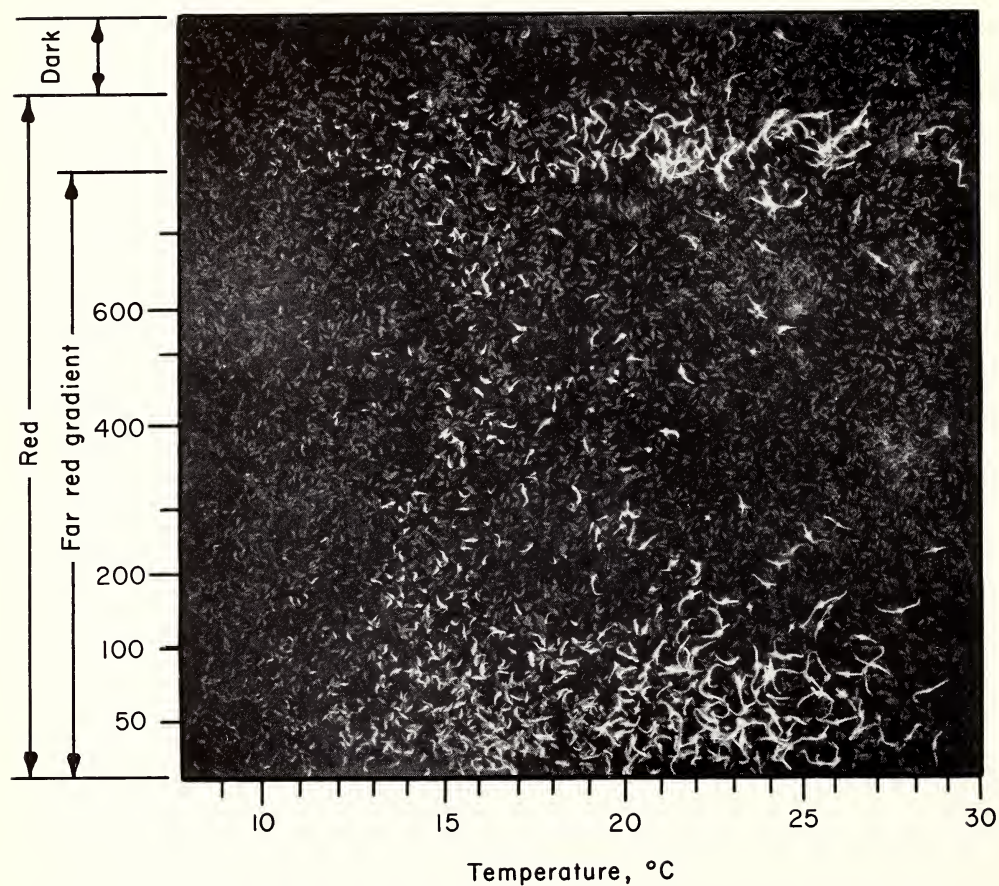


Fig. 18. Photograph of the seeds after germination. The gray seeds did not germinate. The white lines are roots. The red light (15 min) was of equal intensity over the whole plate below the dark strip, whereas the far red (1 min), given after the red, was graded in intensity. The intensity units are white-light intensity in foot-candles with the filter removed.

bleaching of each of the bacteriochlorophyll bands.

Extraction of the carotenoids from the chromatophores did not affect the reversible bleaching of the bacteriochlorophyll after the addition of oxidant. Irreversible bleaching, by red light, of bacteriochlorophyll in the chromatophores was found to be little if at all influenced by the extraction of carotenoid.

scopic algae in a crossed gradient of temperature and light intensity. It seemed likely that such a crossed-gradient principle might be useful for seed-germination experiments.

The effect of the interaction of light and temperature on the germination of the seed of *Lactuca sativa* Linn. var. Grand Rapids has been studied in great detail by previous workers. This lettuce variety was

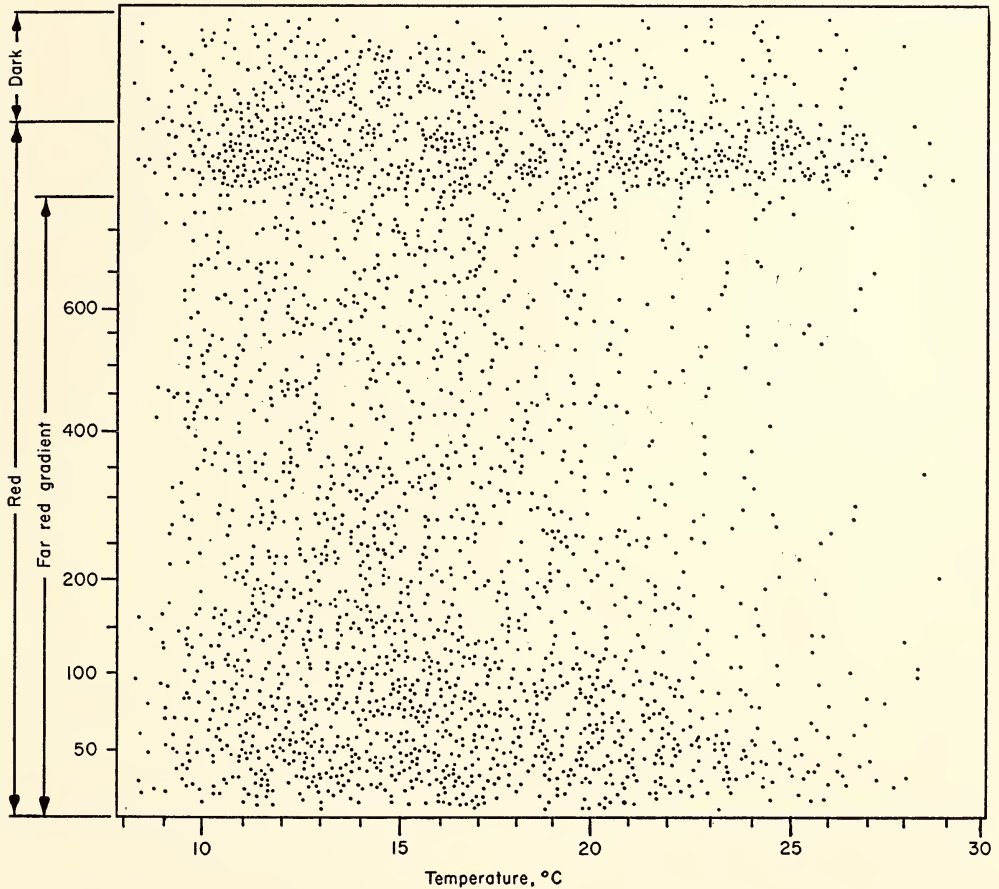


Fig. 19. Summary of three experiments. Each dot represents a germinated seed.

*Germination of Light-Sensitive Lettuce
Seeds in Crossed Gradients of
Temperature and Light*

Ruth F. Elliott

In Year Book 55, pages 261-265, there is described a method for growing micro-

recommended by Dr. E. H. Toole and Dr. Sterling B. Hendricks as suitable for testing the utility of the crossed-gradient plate. It is very light-sensitive, with a greater percentage of germination in light than in darkness. The photoresponse varies with the temperature and the type

of radiation, red promoting and far-red inhibiting the germination of water-imbibed seeds. Because the inhibition reaction is more temperature-sensitive, it was decided to cross a temperature gradient with a far-red radiation gradient. A series of experiments was run in which imbibed seeds were first promoted to full germination by exposure to red light and then exposed to a gradient of far-red radiation. The seeds were left to germinate in a temperature gradient. Also a dark and a red-light control strip were run at the back of the plate covering the entire temperature range.

The general pattern of the response to these conditions is shown in the photograph of the growth chamber (fig. 18) and in the summary of three experimental runs (fig. 19). The results agree well with the type of response found by previous workers. They show that germination in the dark increases with decrease in temperature, and that red light promotes the seeds to full germination over a temperature range of 10° to 26° C. Inhibition by far-

red radiation reduces the germination percentage to near the dark germination level at maximum dosage, the amount of inhibition increasing with the far-red radiation dosage and with the temperature. Superimposed on the germination pattern is a well marked growth-rate pattern which is also temperature-dependent but not light-dependent (fig. 18). The difference in appearance of figures 18 and 19 is due to this fact.

This work demonstrates that the crossed-gradient principle can be applied successfully to seed-testing experiments. Its main advantage over other techniques is the ability to cover a wide range of conditions with one relatively simple experiment. The crossed-gradient apparatus gives the germination response in a two-dimensional picture which could be obtained otherwise only by a large number of individual tests. The results are qualitative rather than quantitative and would be of particular value in testing the range of sensitivity of unknown seeds to two variables.

BIBLIOGRAPHY

- Ahrne, I. See Smith, James H. C.
 Baker, H. G. Genecological studies in *Geranium* (Section Robertiana). General consideration and the races of *G. purpureum* Vill. *New Phytologist*, 56, 172-192 (1957).
 Benitez, A. See Smith, James H. C.
 Clausen, Jens. The function and evolution of ecotypes, ecospecies and other natural entities. In *Systematics of Today*, pp. 139-143. Almqvist & Wiksells, Uppsala, 1958.
 Clausen, Jens, and William M. Hiesey. Phenotypic expression of genotypes in contrasting environments. In *Scottish Plant Breeding Station Report*, pp. 41-51, 1958.
 Clausen, Jens, and William M. Hiesey. *Experimental studies on the nature of species. IV. Genetic structure of ecological races*. Carnegie Inst. Wash. Publ. 615, vii + 312 pp. 1958.
 French, C. S. Review of *A Manual of Paper Chromatography and Paper Electrophoresis*, by Richard J. Block, Emmett L. Durrum, and Gunter Zweig. *AIBS Bull.* 8, 35 (1958).
 French, C. S., James H. C. Smith, and Hemming I. Virgin. Fluorescence spectra of protochlorophyll, chlorophylls *c* and *d*, and their pheophytins. In *Research in Photosynthesis*, pp. 17-18. Interscience Publishers, New York, 1957.
 French, C. S. See also Lynch, Victoria H.
 Giese, A. T. See Smith, James H. C.
 Halldal, Per. Action spectra of phototaxis and related problems in Volvocales, Ultragametes and Dinophyceae. *Physiol. Plantarum*, 11, 118-153 (1958).
 Halldal, Per. Pigment formation and growth in blue-green algae in crossed gradients of light intensity and temperature. *Physiol. Plantarum*, 11, 401-420 (1958).
 Hart, R. W. See Smith, James H. C.
 Hiesey, W. M. See Clausen, Jens.
 Kupke, D. W. See Smith, James H. C.
 Landolt, Elias. Physiologische und ökologische Untersuchungen an Lemnaceen. *Ber. schweiz. botan. Ges.*, 67, 271-410 (1957).
 Latimer, Paul. Apparent shifts of absorption bands of cell suspensions and selective light scattering. *Science*, 127, 29-30 (1958).
 Loeffler, J. E. See Smith, James H. C.
 Lynch, Victoria H., and C. Stacy French. β -Carotene, an active component of chloro-

- plasts. *Arch. Biochem. Biophys.*, 70, 382-391 (1957).
- Lynch, V. H. *See also* Strehler, Bernard L.
- Shibata, K. *See* Smith, James H. C.
- Smith, James H. C., D. W. Kupke, J. E. Loeffler, A. Benitez, I. Ahrne, and A. T. Giese. The natural state of protochlorophyll. In *Research in Photosynthesis*, pp. 464-472. Interscience Publishers, New York, 1957.
- Smith, James H. C., K. Shibata, and R. W. Hart. A spectrophotometer accessory for measuring absorption spectra of light-scattering samples: spectra of dark-grown albino leaves and of adsorbed chlorophylls. *Arch. Biochem. Biophys.*, 72, 457-464 (1957).
- Smith, James H. C. *See also* French, C. S.
- Sorokin, Constantine. Changes in photosynthetic activity in the course of cell development in *Chlorella*. *Physiol. Plantarum*, 10, 659-666 (1957).
- Strain, Harold H. Chloroplast pigments and chromatographic analysis. Thirty-Second Annual Priestley Lecture, March 24-28, 1958. The Pennsylvania State University, University Park, Pa., 1958. vii + 180 pp.
- Strehler, Bernard L., and V. H. Lynch. Absorption spectrum changes in *Chlorella* and the primary process. In *Research in Photosynthesis*, pp. 89-99. Interscience Publishers, New York, 1957.
- Strehler, Bernard L., and Victoria Lynch. Studies on the primary process in photosynthesis. II. Some relationships between light-induced absorption spectrum changes and chemiluminescence during photosynthesis. *Arch. Biochem. Biophys.*, 70, 527-546 (1957).
- Venkatesh, C. S. A cyto-genetic and evolutionary study of *Hemizonia*, Section Centromadia. *Am. J. Botany*, 45, 77-84 (1958).
- Virgin, Hemming I. *See* French, C. S.

DEPARTMENT OF EMBRYOLOGY

Baltimore, Maryland

JAMES D. EBERT, *Director*

CONTENTS

	<i>page</i>
<i>Introduction</i>	309
<i>The Gametes</i>	311
Sperm-cell models and the problem of rhythmic motility	311
Mechanisms of implantation of the ovum	313
<i>Differentiation and Morphogenesis in the Human Embryo</i>	317
Formation of axial structures	317
Morphogenesis of the heart in relation to blood flow	317
Development of the brain	318
<i>The Neural Crest and Its Derivatives</i>	319
A comparative study of the development of the optic primordium	319
Melanogenesis	319
<i>Development of Adrenals and Sympathetic Paraganglia in Madagascar Insectivores</i> ..	320
<i>Chemical Basis of Morphogenetic Movements</i>	321
The chemical bonds between animal cells; a mechanism for type-specific association ..	321
Morphogenetic movements in cardiogenesis	322
Morphogenesis in the cellular slime molds	325
<i>Synthesis and Localization of Tissue-Specific Proteins</i>	326
Development of a quantitative immunochemical approach	326
Immunochemical study of tropomyosin	332
Synthesis of actomyosin and myosin in the regenerating salamander limb	333
Studies on the development of avian hemoglobin	336
<i>The Acquisition of Biological Specificity</i>	337
Induced aspermatogenesis	338
Acquired tolerance to organ extracts	341
An immune reaction: "graft-against-the-host"	343
Organ-specific coordination of differentiation and growth	346
A humoral factor regulating organ regeneration	347
<i>Effect of Hormones in Developmental Systems</i>	349
Sex differentiation	349
Response of the mesenchymal tissues of the guinea pig to estrogens	350
Chemodifferentiation of the visual pigments	356
Protein synthesis in the <i>Cecropia</i> moth	361
<i>Teratogenesis</i>	364
The anatomy of clubfoot	364
<i>Anatomy and Physiology of the Uterus</i>	364
Phases of the menstrual cycle and their interpretation in terms of the pregnancy cycle	364
<i>Anatomy and Physiology of the Placenta</i>	365
Pressure gradients controlling placental circulation	365
<i>Apparatus and Techniques</i>	367
Injection of the blood vascular system of the uterus	367
<i>The Collection of Human Embryos</i>	367
<i>Contributions to Embryology</i>	367
<i>Staff Activities</i>	367
<i>Bibliography</i>	369
<i>Personnel</i>	371

INTRODUCTION

Among the problems discussed in the Department of Embryology during the year, none is more important than the relation between fundamental research and what the late John C. Merriam, third President of the Carnegie Institution of Washington, called organization of knowledge. Not only the investigator, but also the public, is quick to recognize the value of intensive research in reaching into the unknown and making available new facts, contributing to the increase of information. Frequently less appreciated are advances resulting from the organization of knowledge, which, as Dr. Merriam put it, leads to "such glimpsing of relations between areas of knowledge as makes possible the formulation of great generalizations or principles." Understanding and greater perspective are advanced by the complementary interaction of both types of creative endeavor. Twenty years ago, President Merriam concluded that the most effective research organization (and the most economical scheme of operation) is one in which there is a balance between activities devoted to intensive research and those giving vision over larger areas.

In reaching toward this goal, the Institution's policy of cooperation in research has been an added source of strength. The situation of the Department of Embryology, in close association with the Johns Hopkins University, has been particularly fortunate. In coming to a decision on the site for the new physical plant proposed for the Department of Embryology we have reaffirmed our belief that this community of cooperative endeavor and interest is of mutual advantage. At the invitation of the Johns Hopkins University, the Institution and the University have begun discussions about establishment in the near future of the Department of Embryology in new quarters to be built by the Institution on a wooded

site at the northwest corner of the Homewood campus of the University.

This decision recognizes two changes that have occurred in the evolution of the Department of Embryology, a gradual shift in emphasis toward basic research in chemical and physical biology, and a growing awareness of greater responsibility in training the embryologists of the future. Readers of these Year Books have recognized that the current emphasis on research by graduate students departs from a long-standing tradition. Although many young people received a year's training, few students had been encouraged to seek advanced degrees based on research carried on in the Department. We believe that the Institution should not assume teaching functions in a formal sense, i.e., should not grant degrees in any sort of course. On the other hand, we hope to continue, in cooperation with the Johns Hopkins University, training promising students in the field of embryology. Every university believes that its graduate students are a highly selected group; thus it may seem redundant to emphasize that the program of the predoctoral fellows in the Department calls for students who are unusually creative and independent. The decision to encourage such students springs not only from the interest of the Director in graduate training but also from the awareness of the entire staff of the urgent need for young men and women keenly interested in problems of development. It is our belief that strongly motivated, enthusiastic students can make a lasting contribution to the research program of the Department, a belief supported strongly by the progress of students now in residence, recorded in this report. It should be emphasized that this plan does not replace, but rather complements, the program for postdoctoral fellows, which is being continued.

In view of the discussions, field trips,

and general air of excitement generated by the preliminary stages of planning for the new building, it can hardly be said that the past year was one of uninterrupted reflection and research. Nevertheless, augmented by one major appointment, the staff continued to work vigorously in the several lines of research already established and initiated experiments aimed at opening up new pathways.

Dr. Mary E. Rawles became a member of the Department on October 1, 1957. Long associated with Professor B. H. Willier, Dr. Rawles is known as an experimentalist *par excellence*. Her interests in the heart-forming areas of the early embryo, in myogenesis, and in the neural crest and melanogenesis are closely related to the new investigations of other members of the group. In view of her well known ability to transmit her enthusiasm and skill to others, it may confidently be expected that the competence of the Department to deal with these problems will be increased. In addition to her experimental program, to be discussed later in this report, Dr. Rawles has assumed responsibility for the Collection of Human Embryos as Curator.

Six Visiting Fellows contributed to the research program of the Department. Dr. William E. Adams, Professor of Anatomy in the University of Otago School of Medicine, Dunedin, New Zealand, began a five months' stay in October 1957. Professor Adams, a skilled comparative anatomist and embryologist, centered his attention on the Bluntschli Collection; his important findings on the development of the adrenals and sympathetic paraganglia in insectivores will be described below. Doctor Adams' research and travel were supported by a grant from the Carnegie Corporation of New York.

Although Dr. Louis E. DeLanney, Professor of Biology at Wabash College, completed his appointment as Fellow of the Carnegie Institution of Washington in September 1957, his association with the Department is by no means ended. He

and Dr. Ebert are continuing to carry on a valuable exchange of criticism and information as they prepare the results of their long-term collaborative experimental study of the developing spleen for publication in volume 37 of the *Contributions to Embryology*.

On March 15, 1958, Dr. Seymour Katsh took up his duties as Assistant Professor of Pharmacology in the University of Colorado School of Medicine, thus terminating an unusually productive two and one-half years' program conducted as Fellow of the Population Council. Dr. Katsh's analysis of the phenomenon of induced aspermatogenesis, carried on in consultation with Dr. David W. Bishop, yielded many thought-provoking results.

Dr. Hans Laufer, who first joined the Department in the fall of 1957, has been reappointed a National Research Council Fellow in the Medical Sciences to continue his studies of protein biosynthesis during differentiation and growth in the regenerating salamander limb and the *Cecropia* moth. Dr. Jacques Mulnard, Fellow of the Rockefeller Foundation, completed his research in collaboration with Drs. R. F. Ruth and James D. Ebert in the summer of 1957, and returned to his research and teaching position in the Laboratoire d'anatomie et d'embryologie humaines, Université de Bruxelles. Dr. Malcolm S. Steinberg, whose provocative ideas contributed importantly to the program of the Department during the past two years, completed his appointment as Fellow of the Carnegie Institution of Washington and prepared to assume a new position as Assistant Professor of Biology in the Johns Hopkins University.

Forty-two investigators from twelve countries made visits of several days to a fortnight, to work with the Collection of Human Embryos, to take part in specific experiments in progress, and to consult with members of the staff and observe the program of research. Among those whose studies are sufficiently well developed to warrant special mention are the following:

Dr. Chester H. Heuser, Research Associate of the Department of Embryology, returned to the laboratory to continue his work on early human embryos. Drs. Roy E. Crowder and E. Carl Sensenig each made several visits to the Department, following up their previous studies on the development of the adrenal gland and spinal column, respectively. Dr. Pieter A. DeVries resumed his study of the development of the heart, some of the results of which are described below. Dr. Arthur LaVelle undertook a survey of specimens

suitable for histochemical studies of neurogenesis. Members of the Department continue to take an active interest in the work of Drs. Roger B. Scott and Lawrence R. Wharton, Jr., in the field of experimental endometriosis. Dr. George Settle has again made use of the Collection in preparing his forthcoming paper on the anatomy of club-foot. Professor Emil Witschi renewed his study of sex differentiation in the human embryo, employing the incidence of the sex chromatin body as a guide to the genetic sex.

THE GAMETES

Sperm-Cell Models and the Problem of Rhythmic Motility

In his analysis of the molecular basis of rhythmic motility in spermatozoa, Dr. David W. Bishop has continued to make effective use of glycerine-extracted mammalian sperm-cell models. The investigation of sperm-cell models, which have many of the properties of the extracted muscle fibrils of Szent-Györgyi and Weber, has led to a better understanding not only of the nature of the contractile process in the models but also of the mechanisms of flagellation in normal spermatozoa. The behavior of mammalian sperm models, stimulated into rhythmic motility by the addition of adenosine triphosphate (ATP), suggests (*a*) a self-sustaining cycle of contraction and relaxation under constant external conditions, unlike the single contraction induced in muscle, and (*b*) an inherent synchronization between contraction and relaxation processes within the same cell, if not within the same contractile fibril. When a part of the system is contracting, another part must be relaxing. It is not known whether, in spermatozoa, the ATP-energy source is fed into the contractile system during the contraction or the relaxation phase. Indeed, the problem has not been fully resolved for muscle; but the contraction-relaxation cycle and the factors that affect it can be approached in spermatozoa in

terms of the experience gained from the study of muscle.

In order to contract, muscle must possess a degree of plasticity. ATP contributes to the plastic state, for the depletion of ATP in muscle leads to rigor. Often the plasticizing function of ATP can be replaced by inorganic substances, including pyrophosphate. Hoffmann-Berling has demonstrated that in the model of the tail of the grasshopper sperm cell the frequency of beat is a function, not primarily of ATP concentration, but rather of the concentration of plasticizing agent—given a certain minimal amount of ATP, the frequency may be increased with increasing concentrations of pyrophosphate. Some of Bishop's preparations with mammalian sperm models confirm this. For example, when rat sperm models are washed thoroughly, or are stored overnight at 0° C, their ability to respond to ATP is gradually lessened. The addition of pyrophosphate (5×10^{-3} M) counteracts this loss without necessitating an increase in ATP concentration (10^{-3} M). On the other hand, sperm models prepared from the bull or the rabbit do not respond favorably to the addition of pyrophosphate; sufficient plasticizers are retained in the models, or the added ATP itself serves this function. Of further importance is the fact that neither bull nor rabbit sperm models increase their beat frequency with an in-

crease in the concentration of ATP. The response is the same over a range of ATP concentrations from 10^{-4} to 8×10^{-3} M.

The relaxation stage, and possibly the relaxation phase as well, of muscle is currently regarded as due to the activity of the so-called Marsh factor which can be isolated from rabbit skeletal muscle. Its function is to inhibit contraction, in the absence of free calcium ions, by an action on the contractile protein, on the ATP-ATPase complex, or on some unknown intermediate in the contraction system. It may even act by nullifying the plasticizing effect of ATP. Relaxing factors are now believed to play a role in the movements of cells other than muscle. The work of Hoffmann-Berling suggests that the presence of relaxing factors in dividing fibroblasts *in vitro* may govern the differences in response of the equatorial, polar, and spindle regions under constant conditions of ATP concentration.

Several lines of evidence suggest that the flagellation of spermatozoa is influenced by relaxing factors. For example, Bishop has found a beneficial effect of trace amounts of calcium (a divalent cation unnecessary for muscle model activity) in the reactivation of bull and rabbit sperm models. Moreover, motility in the bull sperm model is inhibited by the addition of an exogenous relaxing factor prepared from rabbit muscle by the method of Portzehl. The inhibition occurs at supraoptimal concentrations of ATP and only in the absence of free calcium ions. The inhibition is counteracted and motility is regained by the addition of Ca^{++} . Excess Mg^{++} cannot substitute for Ca^{++} in this reaction. Bishop has suggested that at high concentrations of ATP the system is very close to being supersaturated with substrate, in this case ATP. In the absence of Ca^{++} , the Marsh factor combines with contractile protein (ATPase) and removes enough of it from the system to increase the relative amount of ATP, thus leading to substrate inhibition. The addition of Ca^{++} removes or dis-

sociates the Marsh factor and restores the original balance between ATP and ATPase. This argument is supported by the observation that the addition of Versene (ethylenediaminetetraacetic acid, EDTA), even in an excess of Mg^{++} , inhibits motility of the models, most likely by the removal of all free Ca^{++} .

The studies on extracted sperm models have provided clues to the nature of the flagellation process in normal cells. The movement of spermatozoa and of other flagellated cells has been described previously, with the aid of cinematographic recording, as either a simple two-dimensional beat or a three-dimensional spiral wave. Conflicts in interpretation of various investigators, including those working on the same species of sperm, have been compounded by the possibility of the conversion of a two-dimensional motion of the tail into a three-dimensional forward spiralization of the entire sperm because of the nonsymmetrical configuration of the cell, particularly of the sperm head. For example, Bradfield, basing his speculation on early electron micrographs of sperm tails which indicated a radial symmetry of the longitudinal fibrils, assumed the beat to be a spiral pattern produced by successive waves of contraction originating at the base of adjacent fibers. Such coordination necessitates an ionically intact and irritable organization that is most certainly destroyed in cell models, which, however, continue to flagellate. More recent electron micrographs of sperm tails demonstrate not merely a radial arrangement of fibrils but a superimposed bilateral symmetry based on fibril diameter. Assuming that the fibrils are the contractile elements, this finding suggests either a two-dimensional wave or a beat that is stronger in one plane than in any other.

From his studies of motility of reactivated cell models and of living sperm, Bishop argues that both two- and three-dimensional movements can and do occur in the same cell at the same time, that

certain environmental and physiological conditions may favor one or the other, and that one or the other type of motility can be reduced or eliminated by alterations in the experimental media surrounding the cells. The treatment associated with glycine extraction divests the cells of their normal properties of irritability and permeability. Simultaneously they lose their ability to beat in a coordinated, spiral manner. Such cell models beat rhythmically but only in one plane. Their only forward movement is slow and jerky, and occasionally they even move backward. Nevertheless, the two-dimensional beat is vigorous and may exceed in frequency that of the normal unextracted sperm. These extracted cells have thus retained the mechanism for the rhythmic contraction-relaxation cycle but have lost the coordinating mechanism that normally provides for a contraction sequence in adjacent fibrils.

Unextracted spermatozoa, on the other hand, cells with relatively long and flexible tails such as sperm of the common squid *Loligo*, can be slowed down by dilution of the sperm mass or by the gradual depletion of ATP and the substrates that permit its reconstitution. This treatment, in a sense, uncouples the two kinds of sperm motility. Stationary cells and even those drifting backward in the surrounding medium can rotate rapidly about their longitudinal axes, owing to a spiral flagellation of the tail elements. The frequency of this type of fibrillar motion is high, and, for any given sperm, the direction of rotation remains constant. The second, more violent but less rhythmic beat in these retarded sperm is a two-dimensional lashing of the tail. This contraction-relaxation cycle has a time constant distribution of about 3 to 1, with a definite pause intervening between contractions. The wave motion is restricted to the sperm midpiece and anterior end of the tail and, as far as can be determined, is always in a plane parallel to the mitochondrial spur, a structure attached at one side of the head of the squid sperm. Bishop, in collaboration with

Drs. B. Afzelius and R. Kane, of the Department of Biophysics, the Johns Hopkins University, is currently studying the relation of the spur to the bilateral symmetry of the tail filaments as seen in electron micrographs. The experimental separation of the contraction-relaxation mechanisms from the coordination mechanisms raises a number of provocative questions; the analysis of flagellar motion using these techniques clearly constitutes a problem demanding concerted attention.

Mechanisms of Implantation of the Ovum

Implantation, or the attachment of the egg to the uterus, becomes more understandable when it is regarded, not as a single event, but as a definite sequence of events embracing the following mechanisms: uterine muscular, by which ova are transported to and located at their sites of implantation; adhesive, by which the eggs are held fast to the uterus; and invasive, by which the trophoblast of the ovum grows into the uterine tissues to form the placenta.

Dr. Bent G. Böving's immediate objective is to explain each mechanism in terms of its stimulus, its effector, and its results. A sequential list of mechanisms and their components (table 1) has been giving preliminary service for several years by pointing out what remains to be investigated in the "pilot" species, the rabbit. It has begun to give interim service as a basis for comparing implantation mechanisms in different species, for example, clarifying aspects of implantation in man and the monkey that have not been understood previously. It is intended to give ultimately a systematic and comprehensive understanding of implantation, and it has progressed in this direction far enough so that the simple list has acquired a new dimension: it can be read vertically. Doing so (with due regard also for details not tabulated) reveals that each mechanism depends on a previous one and sets the stage for a succeeding one, and, when the time comes, yields to

it. Thus, the continuing exploration of the separate events must be accompanied by the search for the mechanisms by which they are integrated.

Studies pioneered largely by Dr. George W. Corner proved that the hormone progesterone from the corpus luteum of the ovary is necessary for implantation. Since many mechanisms are involved in im-

has not been found, and, for all we know, may not exist. The most practical effort, which may eventually contribute toward a unified answer, appears to be further exploration of the manner in which progesterone may affect each mechanism.

Muscular mechanisms. After the ova have entered the uterus and developed into blastocysts, their transport is almost cer-

TABLE 1. Mechanisms of Rabbit Blastocyst Implantation

DAYS AFTER MATING	TYPE	STIMULUS	EFFECTOR	ACTION	ANATOMICAL RESULT
3-5	MUSCULAR	"spontaneous"	muscle	well-propagated contraction	blastocyst transport with random arrangement
5-7		P distention	P muscle	decreasingly propagated contraction	blastocyst transport with "even" spacing
6½-7		P distention	P muscle	dome + grasp	stop transport, flatten endometrium, hold blastocyst antimesometrially
7	ADHESIVE	P alkalinity	?P glaiolemma P mucolemma ?	adhesion	attachment (abembryonic, hence orients blastocyst)
7		(trophoblast knobs	digestion ?	disintegration of abembryonic mucolemma and glaiolemma
7		P alkalinity	trophoblast	adhesion	attachment (over vessel, thus "aims" invasion)
7-9	INVASIVE	P alkalinity push ?	uterine epithelium trophoblast knobs	epithelial dissociation trophoblastic penetration	invasion
				(anchor exchange	shedding remains of lemmas) temporary (yolk sac) placenta
8½-10		?	trophoblast (embryonic pole)	?	invasion
				anchor exchange	definitive placenta

plantation, it is natural to inquire which of them are progesterone-dependent. Finding several to be so dependent (marked "P" in table 1), we are confronted with the difficult question of how a single chemical may coordinate a number of diverse mechanisms some of which operate at different times. Despite such deeply probing studies as those of Corner and Csapo on progesterone regulation of uterine muscle (Year Book 55, pp. 282-284), a unified answer in the sense of a shared mechanism

tainly accomplished by muscular contractions that pass in waves along the uterus. In the monkey and rabbit, however, the waves progress, respectively, about 100,000 and 1000 times as fast as the blastocysts are propelled. Two anatomical features help explain why the rate of blastocyst propulsion is slow:

1. It has been customary to think of blastocysts as suspended in uterine fluid, presumed to fill the uterine lumen seen in the usual histological preparation. But

preparations that minimize distortion (by freezing or by perfusion) show almost no lumen. Moreover, when the uterus of the living animal is opened, the interior is moist but no fluid flows out. The lumen contains little more than a thin film of mucus. It must be in this scant, viscous medium that the blastocyst is carried rather than in a copious, watery fluid. The uterine mucus covers the blastocyst as a membrane, the gloiolemma, deposited outside the mucolemma, a somewhat similar layer secreted around the egg in the tube. Similarities of the secretions and secretory cells and Greenwald's proof that the tubal mucin secretion is regulated by progesterone suggest that the uterine secretion also may be progesterone-dependent, but this possibility remains to be tested.

2. The endometrium, the succulent tissues interposed between the encircling muscles and the blastocyst, must distribute pressures from waves of contraction much as a fluid would, so that, at least while a blastocyst is small, pressures behind it could not differ greatly from those in front and could contribute only slightly to its propulsion. The cushioning effect may be presumed to be increased by the prostational thickening and edema of the endometrium.

Previous studies suggested that, when blastocysts attain a critical size, they stimulate the uterus to produce waves of contraction from each point of stimulation (and from both ends of each uterine horn) which mutually repel the blastocysts until they are equally separated and the forces cancel out. Both the suspected stimulus (blastocyst expansion) and the appropriate behavior of the effector (uterine muscle) were known, from work of Corner, Csapo, Greenwald, and others, to be progesterone-dependent and estrogen-inhibited, but there was no rigorous, direct proof that the size of blastocysts *per se* was the stimulus, or that progesterone in the absence of other peculiarities of pregnancy was sufficient to cause even spacing. This objective appears within reach. In a few

experiments five or six glass spheres inserted in uteri of pregnant or progesterone-treated rabbits have become distributed like blastocysts of the same size, whose normal range was defined by a previous study reported in Year Book 55. In an animal that failed to become pregnant the distribution of spheres was not distinguishable from random. Replication and control experiments are in progress, and it is expected that the blastocyst-spacing study will be completed during the coming year.

Not only does the progesterone-dependent blastocyst expansion turn on the spacing mechanism; it also turns it off. The blastocysts soon become so big that the uterus cannot move them along. Further expansion causes the uterus to balloon out, forming a dome in which the blastocyst is grasped by circular muscle that has been pushed aside into bands during the formation of the dome. The muscular grasp is considered a preliminary attachment which, in addition to functions concerned with blastocyst orientation, probably helps the succeeding adhesive stage much as hand pressure helps adhesion of a postage stamp.

Adhesive mechanisms. Quantitative anatomical analysis showed that the adhesive and invasive attachments of rabbit blastocysts to the uterus were induced only when accessible uterine epithelium had a blood vessel at its base. When silver nitrate solution is passed through the blood vessels a precipitate is formed at the attachment site, a finding that is interpreted as showing that adhesion and invasion depend on a chemical transferred between the blastocyst and maternal circulation. Since the precipitates are dense within blastocysts before and during implantation, occur chiefly in parts of the uterus near a blastocyst, and do not occur in the absence of a blastocyst, the direction of transfer must be from blastocyst to uterus. Several lines of evidence and inference suggest that the substance in the blastocyst is probably a bicarbonate but that during or after passage to the epithelium it is converted to

a carbonate or carbonic acid and then removed by the maternal circulation.

The silver precipitates have been imitated in the empty horn of unilaterally pregnant rabbits when the lumen was first charged with an "artificial blastocyst," consisting of normal salt solution saturated with calcium carbonate and carbon dioxide. An identical reaction was obtained with sodium bicarbonate solution whose strength corresponded to the maximum bicarbonate concentration of blastocysts. A similar but slightly paler reaction was obtained when the uterine lumen was charged with water through which carbon dioxide had been bubbled, but no typical silver precipitates were found in segments of the same uterus that had been charged with sodium chloride or sodium hydroxide solutions. Since silver ion does not precipitate with carbon dioxide, it is concluded that, in the uterine epithelial cells, carbon dioxide was converted to a form that precipitates silver, probably a carbonate. This conclusion is consistent with Lutwak-Mann's biochemical demonstration of augmented endometrial carbonic anhydrase in pregnant and progesterone-dominated rabbits. The absence of precipitates in non-pregnant rabbits and in spayed rabbits treated with estrogen, their presence in spayed rabbits treated with progesterone, and the occurrence of atypical precipitates in animals treated with a carbonic anhydrase inhibitor (Diamox) are also consistent.

It is concluded that, in the rabbit, blastocyst attachment is promoted near vessels by a progesterone-induced increase in endometrial carbonic anhydrase which speeds the transfer of bicarbonate from the blastocyst by catalyzing the formation of carbonic acid, which is removed by the maternal circulation. A by-product of the conversion, a local liberation of alkalinity, is considered the direct cause of attachment, for blastocysts removed from the uterus to an open dish of indicator solution show alkalinity in the region where at-

tachment occurs, whereas immersion in a solution similarly alkaline makes blastocysts sticky all over. In other words, the "aim" for blood vessels which is characteristic of early trophoblast attachments to the uterus is explainable as the sharply localized induction of adhesion by alkali liberated where bicarbonate is transferred and converted.

Invasive mechanisms. The trophoblastic penetration of uterine epithelium which immediately follows adhesion is probably promoted by the same local alkaline reaction. Its location with respect to vessels is similar. This could, of course, be said to be simply a consequence of succeeding the adhesive attachment there, but the relationship is probably not merely passive. That alkalinity causes dissociation of embryonic cells is well known. It was, therefore, ascertained that sodium bicarbonate solution of appropriate strength causes some dissociation of uterine epithelium at implantation time. In this connection, consider that the invading tissue of the embryo, the trophoblast, is peculiar in having many nuclei but no separate cells. This syncytial character has been regarded as a curiosity, but it can now be recognized as precisely appropriate to the function of the tissue; trophoblast cells cannot dissociate in circumstances which cause the disaggregation of the adjacent epithelial cells. This concept of invasion by differential dissociability at high pH is supported by histological appearances and measurements which show that uterine epithelium has, in fact, been lost at invasions. In a rabbit with well developed attachments, invasions were much wider than could be accounted for by the associated pushing aside of the epithelium.

The pushing aside was measured by using blood vessels attached to the epithelium as markers and comparing the mean separation of vessels at invasions with those elsewhere. The vessels at invasions were significantly farther apart. It is conceivable that the difference reflects only a pulling

apart by shrinkage during preparation, but there are good anatomical and mechanical reasons for thinking that the tropho-

blast is extruded or pushed through the epithelium, and this theory is to be a subject for study in the immediate future.

DIFFERENTIATION AND MORPHOGENESIS IN THE HUMAN EMBRYO

Formation of Axial Structures

During the year Dr. Chester H. Heuser, Research Associate of the Department of Embryology, continued his survey of presomite and early somite embryos of the first 24 days of development (horizons i to x). The description of horizon x having been published in volume 36 of the *Contributions to Embryology*, an account of age groups viii and ix is being prepared. The oldest presomite embryos belong in horizon viii, a period marked by significant changes in the axial structures of the embryo—the prechordal plate, primitive node, primitive streak, notochord, and cloacal membrane. The primitive node can be identified in horizon vii and becomes a conspicuous landmark in embryos of group viii. The youngest members of the group reveal a notochordal process extending forward from the primitive node. Although the archenteric canal has not yet appeared, its development is foreshadowed by the arrangement of the nuclei and cytoplasm in the notochordal process. In slightly older specimens there are small isolated spaces in the notochord and a loosening of the cytoplasm. The first appearance of a lumen may occur at any point along the process or in several places simultaneously. A more or less continuous canal with a dorsal opening at the blastopore can be seen in the older embryos of horizon viii.

Plastic sheet reconstructions have been made of two embryos belonging in younger horizons: No. 8299 is approximately 12 days old and belongs in age group v. Except for an atypical orientation of the germ disk and a misshapen yolk sac, it appears normal and is valuable for comparison with other specimens of the group. No. 8360 is a normal embryo

about 13 days old, one of the type specimens of horizon vi.

Morphogenesis of the Heart in Relation to Blood Flow

Dr. Pieter A. DeVries, of the University of California, has made use of the Collection of Human Embryos in a critical re-examination of the early development of the heart in man, his principal interest being the relation of flow within the heart to the morphogenesis of the pump itself. It is Dr. DeVries' hypothesis that the streams within the heart at any given time are a causal force in its morphogenesis. His interest was directed first toward the genesis and effect of the spiral streaming of the outflow tract, between the left ventricle and the branchial arches. In an effort to understand the fluid dynamics of spirals, he studied intersecting streams *in vitro* and found that the direction and nature of the spirals were a result of the angles of intersection of the streams. The angle of junction of the streams perpendicular to the axis of flow determined the direction of the spiral; thus, in the heart, a left dorsal stream junctioning with a right ventral stream produced a clockwise spiral. It was postulated that the spiral resulting from the junction of two streams represented the force which modified the cylinder of plastic "cardiac jelly" in the outflow tract to produce the bulbar septation.

If the hypothesis is correct, it should be possible to demonstrate that neither spiraling nor cushions develop before the development of a right ventricle so located as to produce a stream junctioning with that of a left ventricle. In an embryo in horizon xv, Dr. DeVries was able to follow the spiral septation in a continuous manner

from the hypothetical junction of streams to the division of streams in the fourth and sixth arches, suggesting that a thorough analysis of the early embryo should be rewarding. Accordingly, serial sections of embryos from 7 somites through horizon xxii have been examined. Spiral cushions cannot be identified in embryos earlier than age group xiv, although some embryos in horizon xiii have the barest suggestion of an elevation of cushion material into the outflow tract. Through age groups xiv and xv the bulbar cushions become more and more prominent. The right ventricle and beginning interventricular septum can be recognized by horizon xii. The left ventricle is in a left ventral position, the interventricular canal and right ventricle being in right dorsal positions. The right ventricle flows into the bulbus, which is in a transverse plane, and the bulbus flows into the truncus in a dorsocephalad direction.

From this time forward through horizon xvi the following events occur: Elongation of the bulbus with resulting caudal, and then ventral, migration of the right ventricle, and some caudal rotation of the left ventricle. The right ventricle folds ventrally on the left much like the action of closing a book. In the growth of the ventricles there is a relative foreshortening of the dorsal wall, with the result that there is an apparent migration of the atrioventricular canal toward the right ventricle. During this period the enlarging atria assume a more cephalad position in respect to the ventricles.

Wax plate reconstructions of the hearts of 5 embryos have been made. For each embryo, reconstructions have been prepared of the walls of the heart with colored inlay of the "cardiac jelly," the lumen or cavities of the heart, and the jelly itself. One of these embryos from Saunders' collection is staged horizon xv. The following four are from the Carnegie Collection: embryo 8944, horizon xii, 25 somites, magnification $100\times$; embryo 5923, horizon xii, 28 somites, magnification $100\times$; em-

bryo 8066, horizon xiii, magnification $100\times$; embryo 6502, horizon xiv, magnification $100\times$. These models, together with graphic reconstructions, have been illustrated to show the outside walls and inside cavities from three views, ventral, and right and left lateral. Obviously, little more than this preliminary account can be given in these pages. A comprehensive report is being prepared for publication.

Dr. DeVries has also initiated a comparative study of the development of the interventricular septum. Available for study are an illustrated series of the development of the pigeon heart (7 through 34 somites) prepared by Miss Doreen Davis from specimens in the collection of Dr. George W. Bartelmez, and a closely staged series of the heart of a lizard. Attention is being paid to the phylogenetic as well as the ontogenetic development of the interventricular septum, right ventricle, bulbus, and truncus. Preliminary studies of the Amphibia support the idea that there is indeed an interventricular septum present in developing amphibians, throwing doubt on the prevailing concept that modern Amphibia have "lost" their interventricular septum.

Development of the Brain

Dr. George W. Bartelmez and Dr. Anatole S. Dekaban, Head of the Section on Developmental Neurology of the National Institute of Neurological Diseases and Blindness, are collaborating in a study on the development of the human brain. Their principal objective is to trace the development of the major landmarks of the brain in human embryos from horizon xi (2.5-3.0 mm) to horizon xxiii (28-30 mm). Several years ago Dr. Bartelmez analyzed the development of the brain in age groups xi to xiii; with Dr. Dekaban's collaboration, progress has been made in studying horizons xiv through xvii. Dr. Dekaban has assumed responsibility for completing the study of later stages. Further description of the program is deferred to a future report.

THE NEURAL CREST AND ITS DERIVATIVES

A Comparative Study of the Development of the Optic Primordium

Dr. George W. Bartelmez is preparing for publication a second "chapter" of his comparative study of the ontogeny of the optic primordium in mammals. His paper on the proliferation of the neural crest in the forebrain of the rat should be completed late in 1958, for publication in volume 37 of the *Contributions to Embryology*. Evidence of proliferation of neural crest from the primary optic vesicle in the rat confirms Dr. Bartelmez's findings in man. In analyzing the development of the rat brain, he has found it to agree in detail with the identification of the subdivisions of the early neural folds in man. His reconstructions are based on profile portraits of the intact embryos. Divergent interpretations have been based on projection reconstructions prepared on the premise that the base of the brain in early neural fold stages is a straight line, leading to the failure to recognize the prosencephalic-mesencephalic boundary and the proliferation of neural crest from the forebrain. Despite the wealth of evidence amassed by Dr. Bartelmez, critical experimental proof is lacking. Thus the Director is pleased to report that Dr. Bartelmez and Dr. Mary E. Rawles are planning an experimental analysis of the developmental capacities of the rat forebrain in the near future.

Other aspects of the study, dealing with the development of the optic primordium in the insectivore and the pig, have been delayed pending completion of the study, now in progress, of the development of the telencephalon and mesencephalon in man.

Melanogenesis

It is now generally known that patterns of melanin pigmentation in the vertebrates arise through constant interactions between the pigment cells and their tissue substrates. Any change in the physiological condition of the substrate can bring

about a differential response in pigment cells of a particular genotype.

Dr. Mary E. Rawles has begun to study the distribution of pigment cells during ontogeny in an attempt to correlate changes in their distribution with changes in the physiological properties of the integument of different regions of the body. She has adopted the comparative approach, using the Silver Campine chicken as a representative bird and the black hooded rat as a representative mammal. Both are especially suitable in that each displays striking regional differences in pigmentary pattern; their patterns, furthermore, are relatively stable and predictable. The evidence available indicates that the underlying principles involved in the development of patterns of melanin pigmentation are remarkably similar in these two groups of animals.

In attempting to detect prospective pigment cells and to follow their early distribution in the embryo, various histochemical and metallic impregnation techniques are being tested, in addition to the "dopa" (dihydroxyphenylalanine) reaction. Some progress has been made in recognizing melanocytes in the prospective skin of the dorsal mid- and hind-brain regions of the Silver Campine embryo, at approximately 72 hours incubation, after treatment with dopa.

If prospective pigment cells invade all regions of the body of an embryo, as the experimental evidence would seem to indicate, the question of the ultimate fate of many of these cells arises. Do they move out of areas in which conditions are not favorable or "appropriate" for their further differentiation; do they degenerate *in situ*, or do they remain in an undifferentiated state? Systematic investigations along the lines indicated above may shed some light on these questions.

The fact that pigment cells can be followed without special staining techniques, after the synthesis of melanin granules be-

gins, makes it highly probable that much information about their regional differentiation can be obtained from a systematic study of cleared specimens *in toto* and from cleared whole mounts of skin from various regions of the body at different developmental stages. For this purpose a closely timed series of embryos of the Silver Campine breed has been collected and fixed for study. The investigation should reveal many interesting facts about the development of the down-feather pattern, which to date has been grossly neglected.

Of importance as background information for the present topic is the problem of the organization of the neural crest, the source of the melanoblasts, and its differentiation into various specific cell types. At the present time it is not known whether the cells of the neural crest are pluripotent from the beginning and differ-

entiate according to their later positions or whether their potentialities are already fixed before migration begins.

Dr. Rawles is preparing a manuscript on the development of regional pattern in the plumage of the Silver Campine fowl. During the year covered by this report she brought to completion one major undertaking, a chapter entitled "The integumentary system of birds" to be incorporated in *The Biology of Birds* edited by Professor A. J. Marshall of London. In addition she prepared for publication an article, "Feathers," for the McGraw-Hill Encyclopedia of Science and Technology, a manuscript from data obtained by Drs. T. Seno and I. BüyüKözer entitled "Cartilage formation in somite grafts of the early chick blastoderm," and a paper by T. Seno, "An experimental study on the origin of the ventrolateral body wall of the chick."

DEVELOPMENT OF ADRENALS AND SYMPATHETIC PARAGANGLIA IN MADAGASCAR INSECTIVORES

With the support of the Carnegie Corporation of New York, Dr. William E. Adams, Professor of Anatomy in the University of Otago School of Medicine, spent five months in the Department of Embryology. His stay was uncommonly productive. While studying the development of the vessels in the region of the carotid fork in insectivore embryos of the Department's Bluntschli Collection, he noted in an *Ericulus* embryo a peculiar structure at the bifurcation, which at first looked like an aberrant nodule of adrenal cortical tissue, the possibility that it might have been a peculiar parathyroid II being excluded readily by the difference in structure between it and parathyroid IV, which is embedded in the thyroid. The nodule, which proved to be part of a continuous column throughout the neck and thorax, merging eventually with the adrenal gland, excited Adams' curiosity and led him to study it fully. His analysis of the development of this structure in three different species of insectivores has shown

that it is not cortical tissue, but rather an extensive column of precociously developed paraganglionic tissue, lying in close association with the sympathetic chain, such as is found also in the vespertilionid bats.

In the tenrecid insectivores the adrenal cortex develops from the coelomic epithelium close to the developing gonad, with which it is for a time in contact. The medullary (paraganglionic) tissue arises precociously from the protosympathetic, invades the cortical primordium very early, and is freely continuous with the large preaortic paraganglia, which may be fused across the aorta (e.g. in *Centetes* and *Hemicentetes*). Whereas the paraganglionic tissue (medullary and extramedullary) differentiates rapidly, the cortex remains quiescent until just before birth, forming a cap of small cells over the paraganglionic tissue. Contributions from the sympathetic system continue, but the fate of these small cells has not been determined.

Furthermore, as in certain bats, so in *Centetes* and *Ericulus*, the paraganglionic tissue is not confined to the adrenal region, but extends alongside the sympathetic chain as a distinctive continuous (*Ericulus*) or discontinuous (*Centetes*) column, the cells of which are as well differentiated as in the abdominal paraganglia. In *Ericulus* this column reaches right to the base of the skull, and sections through the carotid fork show that the structure of the paraganglionic tissue is quite unlike that of the carotid body.

CHEMICAL BASIS OF MORPHOGENETIC MOVEMENTS

The Chemical Bonds between Animal Cells; a Mechanism for Type-Specific Association

When and how does a cell acquire the ability to recognize that a molecule or cell to which it is exposed is foreign, and just how foreign it is? Does every cell of the body have the ability to distinguish like and unlike, or is this ability the property of only a few cell types? Aggregates of intermixed embryonic chick and mouse cells of the same type, e.g. chick and mouse chondrogenic cells, reconstruct a chimeric tissue consisting of interspersed cells of the two species. Aggregates of intermixed embryonic cells of different types become associated according to type, chick nephrogenic cells in one grouping, mouse chondrogenic cells in another. At one stage in development, then, a type of tissue specificity dominates over species specificity; cells aggregate histotypically regardless of generic origin.

Dr. Malcolm S. Steinberg's principal objective, the manner in which such histotypic associations are established, may be stated most cogently in his own words, "We are confronted with the question of how cells are able to discriminate among their fellows. The selective mechanism must reside in the cell surfaces, for it is the surface which either forms or fails to form connections with the surfaces of other cells which are encountered. But, before

Dr. Adams presented his principal findings at the annual meeting of the American Association of Anatomists, held in Buffalo during the spring, 1958, and he has prepared a fully illustrated report for publication in the *Journal of Embryology and Experimental Morphology*. The Director hopes that others interested in comparative embryology will be stimulated by Dr. Adams' success to make use of the Bluntschli Collection, which contains a wealth of valuable materials.

we can discover wherein the selectivity of the cell surface lies, we must first know the general mechanism by which cells are held together. The specificities may then be found in the subtle manipulations of this mechanism by the cells."

In continuing his analysis of the electrophoretic behavior of living chick embryo cells, Dr. Steinberg learned that the surfaces of these cells are strongly acidic, being negatively charged even at pH 4. Ambrose and his collaborators have found that mammalian kidney and liver cells, both normal and malignant, and more recently the cells of a mouse sarcoma, are also negatively charged. The electronegativity of the cell surface focuses attention on calcium, since calcium ions, which are necessary for the cohesion of tissue cells, must be bound to electronegative sites. Steinberg advances the possibility (earlier stated by Coman) that Ca^{++} binds cells together by acting as a direct cationic bridge between monovalent anionic sites on the surfaces of apposed cells. He believes it possible, by assuming differences in the spacing of such Ca^{++} -binding groups on the surfaces of cells of dissimilar types, to account satisfactorily for differences in the affinities for one another of such cells, as well as for certain other aspects of their behavior.

This theory is presented, together with a review of pertinent information from

the literature, in a paper published in the *American Naturalist*. Steinberg suggests that the surface of tissue cells is highly ordered in the tangential direction, the order being reflected in a lattice arrangement of ionized acidic groups which bind Ca^{++} or Mg^{++} . The spacings are peculiar to specific cell types (at specific stages in their differentiation), and are wide enough to preclude the formation of slightly soluble calcium or magnesium salts involving two acidic groups on the same surface. Steinberg suggests that cell-to-cell binding is effected through the formation of slightly soluble salts between adjacent cell surfaces. In testing this mechanism, Steinberg began a study of the interaction of Ca^{++} and embryonic cell surfaces, with reference to the phenomenon of reaggregation of dissociated cells in the presence of Ca^{++} .

It should be possible to prevent reaggregation in the presence of Ca^{++} by lowering the *pH* of the reaggregation medium and thus suppressing the ionization of the negatively charged groups which bind Ca^{++} . It has been found that reaggregation of newt embryo cells in the presence of Ca^{++} proceeds normally from *pH* 9 to 6, but is impeded at lower values until a *pH* of 4 is reached, at which reaggregation is reversibly blocked. This result may reflect the suppression of ionization of Ca^{++} -binding groups, but it may equally well be due to secondary effects of the acidic medium. Experiments in progress will decide this question.

In other experiments it was determined that Ca^{++} concentrations of 10^{-4} *M* or higher promote aggregation, but that concentrations of 3×10^{-5} *M* or lower do not. It was also found that Mg^{++} and Sr^{++} , but not Ba^{++} , can promote at least the beginnings of aggregation in the absence of Ca^{++} , the order of effectiveness being $\text{Ca}^{++} > \text{Mg}^{++} > \text{Sr}^{++} > (\text{Ba}^{++})$. The transition metal ions Co^{++} , Ni^{++} , and Zn^{++} are also capable of initiating (but not maintaining) aggregation in the absence of Ca^{++} .

We are concerned here with the chemical events relating to aggregation which occur at the cell surface when Ca^{++} becomes available. In the above experiments the presence, absence, or degree of reaggregation serves as the index by which the effect of experimental conditions is judged. This approach imposes an uncomfortable gap between the reactions that interest us and the effects that we observe. It also precludes the gathering of accurately quantitative data. Therefore Steinberg is beginning experiments using Ca^{45} , in which it will be possible to measure directly the amount of Ca^{++} taken up by dissociated cells under a variety of conditions. From these experiments quantitative information may be obtained that will allow a kinetic analysis of the reaction between Ca^{++} and those groups in the cell surface which, it is conjectured, by binding Ca^{++} , cause cells to aggregate into tissues. It should ultimately be possible, if this view of cell-to-cell adhesion is correct, to state with a fair degree of confidence the nature of the Ca^{++} -binding groups upon which the adhesion depends.

Morphogenetic Movements in Cardiogenesis

In Year Book 56, a fortuitous observation was described which has led to a redirection of the research efforts and interests of Dr. Robert L. DeHaan. In the course of a study of the mechanisms of spontaneous contractility in the embryonic chick heart, he noted that treatment of embryos *in vitro* with high concentrations of the organic base acetylcholine could prevent normal fusion of the paired cardiac primordia and thus produce "cardia bifida" or double-hearted embryos. The principal findings were described recently in the *Proceedings of the National Academy of Sciences*. Among the further questions that must be considered are the following. (1) What agents other than acetylcholine can produce the effect? (2) What is the action of these agents at cellular and

molecular levels? (3) Why is the anomaly apparently restricted to the heart? (4) What process or processes in normal cardiac development are set awry resulting in the formation of double hearts?

Only 10 to 30 per cent of chick embryos treated with an agent producing maximal numbers of cardia bifida actually develop this anomaly; 30 to 40 per cent are usually relatively unaffected; the remainder frequently are completely disaggregated. If a higher concentration of the agent is used,

cate that acetylcholine can cause the release of protein-bound calcium from the cell cortex, making DeHaan's working hypothesis plausible.

Much confirmatory evidence has been accumulated. It has been possible to demonstrate that cardia bifida can be produced by treatment with chelating agents such as citrate, oxalate, or Versene (EDTA). Furthermore, the effects of these agents can be offset by adding them in the presence of exogenous calcium ions. Figure 1

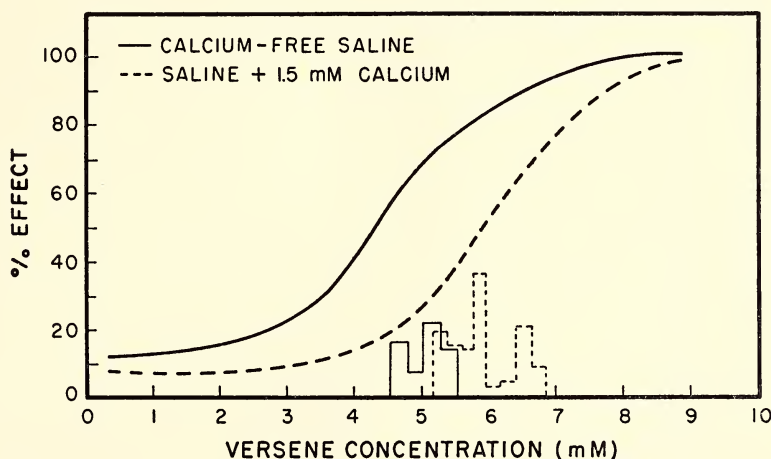


Fig. 1. Dose-response curves obtained by treating groups of embryos with increasing concentrations of Versene. The solid curve shows the percentage of each group which was totally disaggregated or disorganized by Versene dissolved in a calcium-free saline solution. The solid-line histogram shows the percentage of double-hearted embryos produced. The broken-line curve and histogram show the concentrations of Versene needed to produce the same results in the presence of 1.5 millimolar Ca^{++} .

a greater proportion of the embryos are disaggregated. This apparent loss of cell adhesion, noted in some of the first embryos treated with crystals of acetylcholine, suggested that the lack of fusion of the cardiac primordia was the result of a limited dissociation of cells of the endoderm and/or mesoderm, insufficient to have fully disaggregated these tissues, but severe enough to have disturbed the normal cellular associations required for morphogenetic movements. It has been known for half a century or more that cell associations involve calcium ions. Experiments by L. V. Heilbrunn and others indi-

shows a pair of dose-response curves obtained by treating groups of embryos with increasing concentrations of Versene. The solid curve represents the fraction of each group which was totally disaggregated or disorganized by Versene dissolved in a calcium-free saline solution. The solid-line histogram shows the percentage of double-hearted embryos produced. It can be seen that at about 4.3 millimolar Versene 50 per cent of the animals were disaggregated or disorganized. Cardia bifida was produced in the range 4.5 to 5.5 millimolar Versene. When the agent is added to embryos in the presence of 1.5 millimolar calcium ion,

however, the concentrations needed to produce the same results are seen to be 1 to 2 millimolar greater (broken-line curve and histogram). Moreover, if Versene at a concentration of 5 millimolar is added to embryos in saline containing an equimolar concentration of Ca^{++} , no deleterious effects are seen; the embryos are protected completely.

Comparable results have been obtained with citrate ion; its effective concentration, however, is about ten times that of Versene, i.e. 50 millimolar. Since Versene

dependence of the production of double hearts on the calcium-binding strength of the agent used seems clear. Further experiments, however, have suggested that this simple relationship does not obtain fully, since it has been found impossible to offset the teratogenic effects of sodium malate or succinate with Ca^{++} . Studies to clarify this apparent difference are in progress.

Microscopic examination of the double-hearted embryos, fixed and stained as whole-mounts or sectioned serially, has brought to light two striking facts that aid

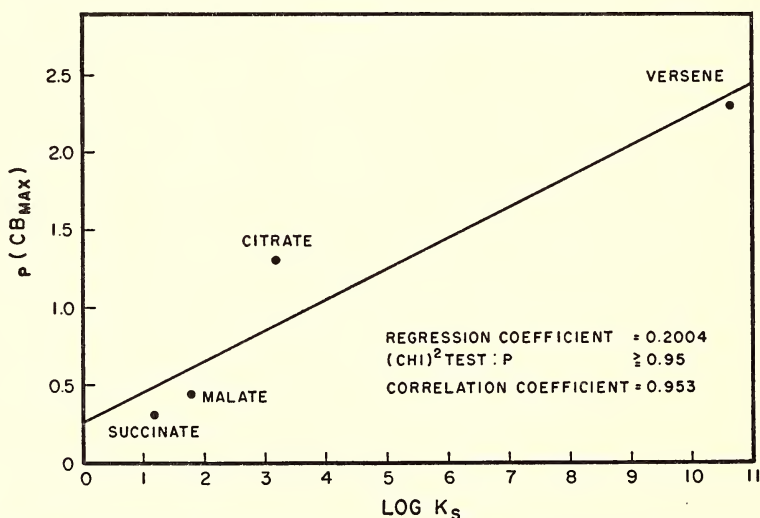


Fig. 2. Correlation between production of cardia bifida and strength of calcium-binding agent.

binds Ca^{++} far more strongly than citrate does, this result is not unexpected. In fact, if the binding of Ca^{++} is indeed the basic causal factor in producing cardia bifida, a series of agents with progressively decreasing calcium-binding strengths should require progressively increasing concentrations to produce similar developmental effects.

Such a series is constituted by the substances Versene, citrate, malate, and succinate. In figure 2 the log of the equilibrium stability constant ($\text{log } K_s$) of each of these compounds with Ca^{++} is plotted against the negative log of the concentration required to produce a maximal percentage of cardia bifida ($p(\text{CB}_{\text{max}})$). The

in interpreting the anomaly at a morphogenetic level: (1) cardia bifida is frequently found in conjunction with some degree of incomplete closure of the neural folds, and (2) in every embryo the presence of double hearts is seen to be associated with some malformation of the foregut. Either there is complete failure of invagination of the endoderm to form the tubular foregut, or such tubulation has begun but the anterior intestinal portal has not regressed as far caudally as it would in an untreated embryo. These facts seem to indicate that the primary action of the calcium-binding agents was to prevent or retard folding movements and tubulation of cohesive epithelial layers,

i.e., the endoderm and neural ectoderm. Studies like those of Ruth Bellairs of the normal movements of the endoderm during foregut formation make clear that it is these movements which carry the mesoderm of the paired cardiac primordia together in the ventral mid-line, allowing formation of the single tubular heart. Prevention of these endodermal foldings would thus necessarily result in the heart-forming areas' remaining in their initial lateral positions.

An attempt to clarify further the relative movements of the endoderm and cardiac mesoderm has been made in a series of carbon-marking experiments carried out in collaboration with Charles J. A. Schulte, III, a senior biology student and undergraduate fellow of the National Science Foundation. More than 100 chicks were marked on the endodermal surface, the movements of the carbon particles being observed and photographed at frequent intervals during the formation of the heart. The embryos were fixed and sectioned serially or prepared as whole-mounts. A preliminary analysis tends to confirm the idea that the cardiac mesoderm remains in contact with the same areas of endoderm from the time of onset of headfold formation to the time of cardiac fusion, that is, throughout the period of early morphogenesis of the heart and foregut.

Morphogenesis in the Cellular Slime Molds

Dr. Robert L. DeHaan's studies on morphogenetic movements involving cohesive cell layers and disturbances of cellular associations have emphasized the compelling need for greater knowledge of mechanisms of cell adhesion and cell movement. The means whereby cells adhere to one another or to some substratum, and the factors affecting the several types of movements that they undergo, remain obscure.

The analysis of some of these problems in the vertebrate embryo is, of course, possible, as the experiments of DeHaan and

Steinberg testify. A simpler system, however, is needed for the attack on certain questions, a system of cells with definite and reproducible adhesive characteristics, undergoing no major histogenetic changes, and easily isolated and maintained in the laboratory. It would appear that these requirements are met by the cellular slime molds (Acrasieae). During its life cycle this primitive form goes through a vegetative stage in which it exists as individual ameboid cells, the myxamebae, which wander freely on the substratum feeding on bacteria. Under the influence of the hormonal substance known as acrasin, these cells migrate toward aggregation centers where they clump together to form a slug-like pseudoplasmodium. During this aggregative phase, according to B. M. Shaffer, their surface characteristics alter so that the cells become "sticky," forming pseudopodial attachments with one another. Only after aggregation into the pseudoplasmodium does recognizable histodifferentiation occur, as the organism enters the fruiting stage and becomes a stalked, spore-forming sporocarp.

In order to learn the techniques of culturing and handling these organisms Dr. DeHaan is spending the summer of 1958 as an Honorary Research Fellow of the Department of Bacteriology, College of Agriculture, University of Wisconsin, in the laboratory of Professor Kenneth B. Raper. It has already been possible to demonstrate that these cells show similarities in their adhesive characteristics to vertebrate tissue cells. The pseudoplasmodium of *Dictyostelium discoideum* Raper, for example, begins to disaggregate within a few minutes when placed in a dilute solution of Versene. The individual cells show very intense lobose pseudopodial activity and surface "bubbling," similar to that described recently by Dornfeld and Owczarzak in cultivated fibroblast cells treated with Versene. In the cellular slime molds, as in metazoan cells, Versene disaggregation is apparently reversible by

flushing with a saline solution containing calcium. The reaggregated cell masses round up and form normal fruiting bodies. It is hoped that continued use of these or-

ganisms for analysis of adhesive and migratory phenomena at a cell level may provide information applicable to developmental processes in the vertebrate embryo.

SYNTHESIS AND LOCALIZATION OF TISSUE-SPECIFIC PROTEINS

Development of a Quantitative Immunochemical Approach

The widespread application of qualitative immunochemical techniques to embryological problems has created difficulties of interpretation due to lack of adequate quantitative information. Since joining the Department of Embryology, Dr. R. F. Ruth has given first priority to a search for techniques for enhancing the antigenicity of substances of biological interest and for their direct, quantitative analysis.

The immunodiffusion techniques, which have been employed effectively by Dr. Hans Laufer in experiments described later in this report, are based on the differential diffusion of the components of either an antigenic mixture or its antiserum or both, in agar or gel, and the visible precipitation that may occur when the diffusing components meet. Modifications include the method of immunophoresis in which an antiserum diffuses into an electrophoretic dispersion of antigen in a long, thin strip of gel. Since the occurrence of a precipitate is determined by several factors, some of which may differ for each antigen-antibody pair, a series of separate bands of precipitate is formed. Theoretically, it should be possible to standardize the occurrence of zones of precipitation from a knowledge of the precipitin reaction, the rates of diffusion, and the concentrations of the reactants. This goal has been achieved in only a few simple systems of purified antigens. The approach has not proved successful, however, for the quantitative estimation of mixed antigens of tissue extracts. Thus, our expanding knowledge of the immunodiffusion characteristics of biologically active substances makes the deficiencies of quantitation, which have been the chronic bane of immunology, more

rather than less acute, particularly in the determination of the degrees of similarity or difference of the components of two tissue extracts.

The demonstration that some of the antibodies to a given antigen are not neutralized or removed by a related antigen is presumptive evidence of intrinsic differences between the two antigens. Sometimes these differences can be expressed quantitatively by adapting the quantitative precipitin technique, as in the well known comparisons of chicken and duck ovalbumins and human and bovine serum albumins, respectively. More commonly such differences cannot be quantified, because of the dependence of the quantitative precipitin technique upon the point of antigen-antibody equivalence. Since each antigen-antibody pair is likely to have its own equivalence point, the similarities and differences between the component antigens of tissue extracts cannot be readily quantified by measurement of precipitates, as in the simpler systems.

The most important generalization in immunochemistry may be the interpretation of antigen-antibody reactions as consequences of the fitting of a part of the surface of the antigen to a part of the surface of the antibody, an interpretation based on the familiar fact that many simple compounds (haptens) can, after attachment to an antigen by covalent bonds, elicit the production of stereochemically specific antibodies. Kinetic studies of the reactions of natural and artificial antigens with their antibodies indicate that the initial fitting of the two surfaces is reversible. Frequently, the reaction does not reach equilibrium because the initial antigen-antibody complex enters into secondary reactions involving the attachment of more

than one antibody molecule to an antigen molecule, leading to degradative changes that become visible as precipitation, agglutination, or hemolysis. The data of most immunochemical analyses are derived from determinations made on such final products. Inasmuch as they do not give a measure of the dissociation constant of the initial combination of antigen and antibody, such data can hardly be a measure of the structural exactness or specificity of the initial combination of antigen and antibody.

There is ample evidence that the several antibodies present in an antiserum are not exactly alike. They combine with different parts of the antigen. Those that fit a particular part of the surface of an antigen vary as to the exactness of the fit. Some antibodies combine with two molecules of antigen, others do not; but precipitation is thought to depend on the presence of divalent antibodies. Hemolysis may depend on the presence of antibodies of a certain size and affinity for other serum proteins. These and other differences among antibody molecules complicate the utilization of the visible reactions as direct estimates of the initial combination of antigen and antibody.

A number of attempts have been made to purify antibodies by adsorption to and elution from insoluble antigens. Frequently either the antibody or the antigen is damaged under the conditions required for elution. That elution of antibodies can occur under innocuous conditions is shown clearly by Talmage's demonstration of the elution of labeled antibodies from erythrocytes by exposure to excess antibody. In general, successful elution seems to depend on the absence or inhibition of the secondary reactions of the antigen-antibody complex. There is no obvious reason to think that the converse adsorption to and elution of an antigen from an insoluble antibody would be more difficult, and there are reasons for believing that it might be more useful.

The degradative changes leading to precipitation of antigen and antibody seem to occur only when the initial complex of one antigen molecule and one antibody molecule can combine with another molecule. Efforts are being continued to attach antibody molecules to an insoluble supporting medium so arranged that no two antibodies can act simultaneously on the same antigen molecule. Such a combination of soluble antigen and insoluble antibody should be dissociable. Simple washing of such a complex might give only an estimate of the mean of dissociation constants, but when the insoluble antibody phase is in the form of a strip or column, so that individual antigen molecules might combine with an appreciable number of antibody molecules in sequence, it should be possible to characterize the antigen in terms of the number, breadth, sequence, spacing, and timing of discrete bands appearing at the end of the strip or column. The mean of the dissociation constants of antibodies to a particular antigen may be the same, but the standard deviation of the dissociation constants, as represented by the breadth of the antigen band, should be greatly decreased. In order to avoid an impractical dilution of the antigens, which might happen during an elution by washing, the elution must be performed by other means, such as electrophoresis. Since the antibody is bound covalently, migration of antigen-antibody complex should not be involved.

These first experiments have met with only limited success. Antigens have been passed through ion-exchange resins containing adsorbed antibody, ionic resins being used because of their relatively great affinity for proteins, including antibodies. The use of ionic forces to bind the antibody in itself presents a knotty problem. Williams and Stone have solved a related problem by adsorbing a refined antigen onto a special cellulose, adsorbing antibody onto this, and finally passing the test mixture of antigens through a column of this

insoluble antibody. The column removed the appropriate antigen from the mixture present in the test extract. The antigen was irreversibly adsorbed by the column, but in this case each antibody molecule is part of an irreversible complex before the final adsorption of antigen. These results suggest that it may be necessary to consider more carefully the chemical and mechanical structure of the supporting medium.

The requirements for antigenicity are not fully understood. We know that most antigenic proteins are relatively large, stable molecules, containing aromatic amino acids. We know also that antibodies to small molecules can be elicited by injecting antigens to which the small molecules are attached. The part of the small molecule farthest removed from the antigen to which it is attached frequently is the most effective part in determining the specificity of the antibodies evoked. Most of the haptens that have been used are of little biological interest. Aromatic compounds and carbohydrates seem to be particularly effective haptens. The aliphatic hydrocarbons seem, in general, to be much less effective. The choice of a protein carrier for these small molecules seems to be relatively unimportant provided that it is a good antigen in some species. Even homologous proteins can be employed after attachment of a hapten. It is suggested that proteins which are poor antigens lack some relatively simple physicochemical characteristics that might be supplied by attachment to a good antigen by covalent bonds. A method of linking two proteins might provide a basis for the linking of antibodies to an insoluble supporting medium as well. In this sense, the problem of quantification and the problem of antigenicity become one.

What are the requirements for the linkage of proteins? The carrier protein (C) and the nonantigenic protein (H) should not be exposed at any time to damaging agents such as organic solvents, high tem-

perature, extremes of pH , and local concentrations of strong acids. The linkage between C and H should be aliphatic, rather than aromatic, so that the linkage itself will not be a strong determinant of the specificity of the induced antibodies. The linkage should be stable *in vitro* and *in vivo*, thus excluding disulfides. It should be formed by a series of selective reactions so that one type of reactive group may be added to C, and another to H, no further reaction occurring until the modified C and the modified H are brought together. This precaution seems necessary in order to avoid the formation of aggregates of C and H, which might have no more immunological relation to the original proteins than fixed or tanned proteins would have. Lastly, the attachment of reactive groups to C and H should be selective for some common constituent of protein surfaces and should not otherwise alter them.

A review of the published work dealing with the chemical modification of proteins leads to several conclusions. It suggests that the free amino groups of the lysine residues, which seem to be common to all proteins, can be modified with a minimum of damage. The attachment of small aliphatic compounds to these amino groups has relatively little effect on the antigenicity of proteins, in contrast to the damage caused by attachment to other groups such as tyrosine, which is a point of attachment for the aromatic diazonium groups commonly used for immunological modifications. Of the kinds of compounds that readily attach to amino groups, some are not particularly selective (isocyanates, epoxides, nitrogen mustards, azides, etc.). Among the more promising compounds are the acylating agents, labile derivatives of carboxylic acids.

The common acylating agents, such as acyl chlorides and anhydrides, are too labile in aqueous solutions and release one equivalent of acid during the reaction with amino groups. Thus an amino group having a pK that corresponds to a pH of

about 10 is suddenly replaced almost *in situ* by an acid the pK of which corresponds to a pH of 5 or less. This sudden change may be responsible for the modification observed after the acetylation of some proteins with acetic anhydride. The product of the reaction of an acylating agent with an amino group is a peptide bond.

Most of the methods of peptide synthesis require the use of organic solvents. There are three methods that are selective for amino groups, do not release strong acids, and employ an acylating agent stable in aqueous media. The best-known method is the reaction of the cyclic carboxy anhydrides of amino groups, a technique applied to proteins by Becker, who showed that an average of more than 100 glycine residues per molecule can be added to bovine serum albumin without producing any alteration in isoelectric point, sedimentation constant, reactivity with anti-(bovine serum albumin) serum, and other characteristics. This reaction proceeds at physiological pH and at moderate temperatures. As the method is applicable only to the attachment of amino acids, and involves polymerization, it has little direct application to immunological problems. It does, however, demonstrate two points: it indicates that cyclic acylating agents may have the desired characteristics of stability in aqueous media and high reactivity toward the lysine amino groups of proteins; and it indicates that the release of an equivalent of weak acid at the site of attachment is not very damaging. In this case the acid whose pK corresponds to a pH of about 6.4 is carbonic.

A method of peptide synthesis, developed about ten years ago by Chantrenne, makes use of the mixed anhydrides of amino acids and monophenyl phosphoric acid. These anhydrides are stable in aqueous media and highly reactive toward amino groups, but they have not been applied to proteins. An equivalent of acid is released in this reaction, but it is the

secondary phosphoric acid whose pK should correspond to a pH of 6.4 or higher. The synthesis of similar mixed anhydrides from the carboxylic acid anhydrides has also been described. A third method of peptide synthesis in aqueous media makes use of the monoacyl derivatives of water-soluble carbodiimides. It is applicable to many carboxylic acids, but unlike the mixed anhydride method is probably inapplicable to α -halogen acids, one of which will be discussed later.

The mixed anhydrides of carboxylic and phosphoric acids are synthesized from the anhydride of the carboxylic acid. Whatever their final form, most acylating agents are synthesized from, and obtain their acylating activity from, the acid chloride or the acid anhydride. There are a few exceptions, such as the monoacyl derivatives of carbodiimides. Since these derivatives cannot be used, it is necessary to synthesize the carboxylic acid anhydride for each mixed anhydride of a carboxylic acid and monophenyl phosphoric acid that is tried. Another solution would be to synthesize the mixed anhydrides from the carboxylic acids, which are commercially available, and a "high energy" form of the monophenyl phosphoric acid. A search was made for derivatives of phosphoric acid that would serve this purpose, but none was found.

The chlorine derivatives of phosphorus seem to be the most common forms of active phosphorus used for syntheses. Chlorine derivatives of organic phosphates are not readily available and are relatively difficult to purify. The use of these and other derivatives of phosphate is complicated since the object is to obtain the monoacyl derivatives of the phosphate, and most active phosphorus derivatives have more than one reactive site. The cyclic phosphates that are known do not appear suitable for the synthesis of monoacyl phosphates. Since di- and triacyl phosphates are known, a special search was made for cyclic oxalyl and mesoxalyl derivatives of urea, para-

banic acid, and alloxan. Such compounds might react with carboxylic acids to yield the monoacyl derivative. No reference to such compounds has been found. One possible obstacle to their synthesis is the difficulty of removing water from phosphoric acids without forming large amounts of the pyrophosphoric acids. If this step can be achieved, the preparation of the oxalyl derivative may not be too difficult since oxalyl chloride is available, can be used in the *cis* form, and forms five-membered rings readily. There may be steric reasons preventing a diacyl phosphate from forming a five-membered ring, although five-membered cyclic dialkyl phosphates are known. Since six-membered rings occur in metaphosphates, there is no obvious reason to suppose that a six-membered cyclic diacyl phosphate is impossible. But though the orientation of oxalyl chloride in solution can be determined easily by its absorption spectrum in the ultraviolet, similar information does not exist for mesoxalyl derivatives.

Certain amides are active acylating agents although they are not commonly used as such. Most, if not all, of these are synthesized from other kinds of active acyl compound. A possible exception is the acylating agent formed by the complex of boron trifluoride with amides, which seems to acylate active hydrogen compounds by elimination of ammonia as the boron trifluoride complex. The heat of formation of this complex from the amide is unknown. The singular aspect of this reaction is that it does not produce an acidic proton but, conversely, eliminates hydrogen. It appears that this approach may be applied to the synthesis of an acylating agent of considerable immunological promise. Before considering this method it is appropriate to discuss the final step in the linking of the carrier protein (C) and the nonantigenic protein (H).

To recapitulate: the objective is to form a linkage by a series of reactions so that one type of reactive group may be added to C, and another to H, and further reaction

will not occur until the modified C and the modified H are brought together. The two kinds of reactive groups must be stable in aqueous media, they must be parts of aliphatic carboxylic acid residues, they must react readily with each other in aqueous media under physiological conditions, and they must produce a stable covalent bond. Reports of the alkylation of the sulfhydryl groups of wool by trimethylene bromide and the alkylation of the sulfhydryl groups of proteins by α -iodo and bromoacetic acids and acetamides were the only promising leads. The halo and sulfhydryl groups appear to satisfy all the presumed requirements. The reaction of α -halo compounds with protein groups, other than sulfhydryl, are based on attempts at exhaustive alkylation by excess halo acid or amide. Sulfhydryl groups already present can be blocked by pretreatment of the protein with free α -halo acid. If they do not react it is a fair assumption that they will not react with bound halo compounds. On the other hand, the presence of sulfhydryl groups in the protein may make it unnecessary to add any artificial ones.

The naturally occurring sulfhydryl groups of proteins are parts of cysteine residues. Any reactive sulfhydryl group should serve as well. The simplest possible compounds, mercaptoacetic acid and bromoacetic acid, were selected for detailed investigations. These react to produce a thio ether bond. Thio ether bonds, such as that in methionine, are relatively stable in nonbiological environments, and they are split *in vivo* only by specific enzymes. The bond produced by α -halo and mercapto acids should be less stable than most thio ether bonds because of the presence of α -carbonyl groups. The choice lies between using carboxylic acids that carry groups in the highly reactive α position and those that carry reactive groups in the less reactive β , or more distant, positions. If the latter did react to produce a thio ether bond, it should be more stable than

that produced by the α groups. The α derivatives have been selected.

The bromoacetic acid residue may be added to proteins by means of the mixed anhydride of the acid with phenyl phosphoric acid. The mixed anhydride of chloroacetic acid and D-riboflavin 5'-phosphate has been synthesized and found to be relatively labile, but since the mixed anhydride with acetic acid is also rather labile some of the lability must be due to the riboflavin. Monoacetyl phosphate is appreciably more stable than acetic anhydride, and the acetyl derivative of phenyl phosphate is more stable yet. The stability of the bromoacetyl derivative has not been studied and will not be studied until after comparable studies of the sulfhydryl compounds have been completed.

Although the mixed anhydride method offers promise for the addition of haloacetyl residues to protein, it does not look promising for the addition of mercaptoacetyl derivatives. The sulfhydryl groups would tend to react with the acylating agents used in the synthesis of the mixed anhydride or with the anhydride itself. Since thio esters are good acylating agents for amino groups, Ruth has postulated that a cyclic thio ester of mercaptoacetic acid might be the ideal way to add the mercaptoacetyl residue to protein. Several possibilities for the synthesis of cyclic five- and six-membered thio esters of mercaptoacetic acid are suggested.

The five-membered cyclic phosgene derivative of mercaptoacetic acid had been reported previously, but the synthesis was difficult and the compound was reported to be unstable. This compound was of particular interest because it is the sulfur analog of the cyclic carboxy anhydride of glycine which reacts rapidly with protein. The six-membered cyclic oxalyl chloride derivative of mercaptoacetic acid has not been described. The only remaining possibility was the six-membered cyclic anhydride that might be formed by two molecules of mercaptoacetic acid. This would be the sulfur analog of 2,5-diketopipera-

zine, the cyclic anhydride of glycine, a compound found under the name of 2,5-diketodithiane and reported to attach readily to casein and ovalbumin. The increase in sulfur content of casein was directly proportional to the decrease in free amino groups. The compound is stable in aqueous media that are not strongly alkaline. The main limitation at present is the synthesis of the compound, which is obtained by fractional distillation of the polymers produced by heating mercaptoacetic acid. The yield is low and is decreased by the presence of acids. The compound must be synthesized from one of the derivatives of mercaptoacetic acid. The first extensive attempt to synthesize it will utilize the mercaptoacetamide-boron trifluoride complex.

In the course of the investigation Ruth has discovered a qualitative test for acylating agents, in the absence of sulfhydryl groups, based on the decoloration of the neutral or alkaline solution of violuric acid, the oxime of alloxan. The absorption bands of violuric acid are 312 m μ and 542 m μ , and the molar extinction coefficients are 13,560 and 48.8, respectively. The acid is discolored by sulfhydryl groups also, probably involving the reduction of the acid, but no test has been made for that reaction. The reaction with acylating agents seems to proceed mole for mole.

The study has been informative in a number of subsidiary ways. One of the acylating agents that seemed attractive at one time because of its reactivity, stability, inability to cross-link or tan, and lack of acid formation was β -propiolactone. Subsequently it was learned that the derivatives of this compound may be far less reactive than the original compound, and that each derivative presents special problems of synthesis. At that point, interest in β -propiolactone waned. Later, in another connection, an explanation was sought for the use of formaldehyde in vaccine preparation, since it is known to cross-link or tan by virtue of the reactivity of aminomethylol groups originally formed

from free amino groups. Such cross linking or tanning should impair the antigenicity of the material under treatment. No explanation was found. Since formaldehyde attacks amino groups, it is suggested that a better way to make vaccines might be to acylate the amino groups with a compound that would not cross-link, thus inactivating the agent without altering its antigenicity, a serious problem in the preparation of some vaccines. An examination of the literature on vaccine production showed that this approach has been approximated by the use of β -propiolactone. The lactone is not known to be specific for amino groups, but its success implies that a study of it and the monoacyl phenyl phosphates may well be in order. The specificity of neither is assured, but they should certainly be better than formaldehyde.

Immediate plans call for the synthesis of 2,5-diketodithiane and bromo monoacetyl phenyl phosphate, to attach the acyl radicals to proteins, to bring the two types of modified proteins together with the non-antigen in excess, to inject the protein complexes if they are formed, and to test the antisera for the presence of antibodies against the nonantigen. The work will be correlated with the continuing studies of the contractile proteins and hemoglobin.

With the prospect of new immunochemical techniques more sharply in focus, immunochemical studies of the cardiac and skeletal muscle proteins have been resumed. In general, the proteins of muscle can be separated into those of the sarcoplasm of the muscle cell which are readily soluble in physiological saline or blood, and those which are less soluble and are found in the muscle fibrils. The less soluble proteins have been studied with increasing intensity in recent years because they appear to be intimately involved in the contraction and relaxation of muscle. For example, actin and myosin can be recombined to form contractile fibers. The soluble proteins are thought to function in the maintenance and restoration of the

contractile apparatus without being an intrinsic part of it.

From the point of view of the immunochemist, the analysis of developing muscle might have begun with the soluble proteins. At least one of the subclasses of the sarcoplasmic proteins is known to contain one or more antigens suitable for quantitative immunoanalysis. Instead, attention was focused first on the development of the cardiac contractile proteins, a system that appears to provide a unique opportunity for the correlation of immunochemical findings with information from the field of experimental morphology.

The techniques for detecting and analyzing reactions between the contractile proteins and homologous antibodies are based on the following characteristics of antibodies: they may inhibit the enzymatic activities of crude or purified extracts of muscle; they may interfere with the contraction of artificial or isolated muscle fibers; antibodies may also interfere with the development or function of a muscle or a muscular organ. It may be possible to detect the adsorption of labeled antisera by the contractile proteins.

Immunochemical Study of Tropomyosin

During the past year Dr. John I. White, of the Department of Physiology of the University of Maryland School of Medicine, has cooperated with Dr. Ruth in a study of tropomyosin of the skeletal muscle of the adult chicken. Tropomyosin was selected as the initial target in a series of quantitative studies of the proteins of the contractile apparatus. It has no known function in contraction, and no known enzymatic activity; the selection of tropomyosin was based on its known chemical characteristics and not on a presumption of its biological role. From the viewpoint of the embryologist, too, tropomyosin is intriguing. Its very name was assigned by Bailey as an indication of his belief that it is a precursor of myosin. More recently it has been argued that myosin may be

composed of a complex of actin and tropomyosin. The evidence is not altogether convincing, in view of the finding that in the development of the heart the synthesis of myosin precedes that of actin. It is clear, however, that a better understanding of the chemistry and development of the tropomyosin molecule is required.

Dr. White has been able to obtain preparations of tropomyosin of a high degree of reproducible homogeneity as tested by boundary electrophoresis and ultracentrifugal sedimentation. This protein is unusually resistant to heat and is not precipitated from aqueous solutions by boiling. These characteristics and its manner of preparation provide tropomyosin with a high degree of empirical definition. Thus it seems to provide an ideal point of departure for immunochemical analysis.

Contrary to expectation, tropomyosin seems to contain an appreciable amount of aromatic amino acid, as judged by its ultraviolet absorption of 276 m μ in neutral solution and 291 m μ in alkaline solution. After his successful preparation of lyophilized tropomyosin, Dr. White discovered that a component absorbing at about 260 m μ (indicating the presence of purine and pyrimidine derivatives) could be removed by dialysis. Thus this protein appears to be distinct from the nucleotropomyosins reported by others.

At present, the investigators are trying to establish the reproducibility of tropomyosin preparations in terms of their ultraviolet absorption and nitrogen content. The establishment of standard ratios will reinforce the immunochemical comparison of tropomyosins between laboratories. Several series of injections of the protein into rabbits have been made, and the antisera are being tested. In contrast to other reports of the antigenicity of tropomyosin, Dr. Ruth has been unable to obtain a strong and reproducible precipitin reaction. It is planned to test the antisera by other means, including a study of the effects of these and other antisera upon the chick embryo *in vitro*, a test system with

which the laboratory has broad experience. This type of test will be accompanied by some of the more standard immunological tests, which vary as to their possibilities for quantitation and biological significance.

Synthesis of Actomyosin and Myosin in the Regenerating Salamander Limb

Dr. Hans Laufer, Fellow of the National Research Council, has devoted a part of his time to the continuation of a study, initiated at Cornell University with Professor Marcus Singer, of the synthesis of the contractile proteins in the regenerating limb of the newt, *Triturus viridescens*.

The regeneration of the limb embodies problems of dedifferentiation, cell and tissue interactions, and differentiation, including protein synthesis. The lower vertebrates, including newts and salamanders, can regenerate extremities. The regeneration of a limb resembles embryonic development in many respects. Cells accumulate at the amputation surface to form the primitive bud or blastema. These cells divide rapidly in what appears to be a regulated way, becoming differentiated and organized into a functional structure until finally an arm or leg much like the original is formed.

Attention was focused upon the muscle proteins actomyosin and myosin. In studying the appearance of these proteins, Laufer has made extensive use of immunochemical agar-diffusion methods which heretofore had not been successfully applied to muscle proteins. There are several indications that muscle proteins of the lower vertebrates differ appreciably from those of the higher forms, particularly the warm-blooded species. Most of the existing methods for the preparation of purified muscle proteins are based on procedures for rabbit muscle and had to be modified for use with proteins of the newt. The fractionated proteins had to meet rigorous criteria for actomyosin or myosin. Artificial fibers of both these proteins were formed, but upon the addition of ATP only actomyosin threads contracted. In viscosi-

metric tests both solutions had rates of outflow similar to those of comparable rabbit proteins, but only actomyosin became less viscous when ATP was added. Both protein preparations had the ability to split ATP. With the muscle proteins of the newt thus characterized, antibodies were prepared against them in a number of rabbits.

Agar-diffusion methods revealed that, despite repeated isolations, "actomyosin" usually contained three components: actomyosin was the slowest migrating component; myosin moved somewhat more rapidly; and a third antigen, which precipitated at the periphery, was believed to be a complex nucleoprotein. It must be presumed, then, that isolation procedures for actomyosin, at least when applied to the newt, yield a mixture of proteins (fig. 3, pl. 1).

Antisera were prepared against myosin isolated by a method devised by DeVillafraña, and were tested in a manner comparable to that for actomyosin. A number of sera contained antibodies for myosin as well as small amounts of actomyosin. Several, however, were prepared that contained only one component and were believed to be specific for myosin (fig. 3, pl. 1).

These antisera were used to detect the appearance of muscle proteins during regeneration, and to note their effect on the living limb by direct infusion into the blastema. Investigating the development of proteins during regeneration of the forelimb Lauffer found that actomyosin and myosin can be detected first within the regenerate at the stage of hand formation, the "palette" stage. Thereafter an upsurge of synthesis of these proteins was observed (fig. 4, pl. 1). The appearance of these muscle proteins coincides with the time of myofibril formation. The inability to detect muscle proteins in earlier stages of regeneration is also of considerable interest. Since a number of these cells are stated to arise from dedifferentiated muscle, the results point toward a relatively com-

plete dedifferentiation of blastema cells. It may be argued that the muscle antigens are dilute and undetectable, but still present. For this argument there is no rebuttal except to point out the extreme sensitivity of the immunochemical procedures, which are capable of detecting the minor nucleoprotein component in all cases in which one would predict its presence. This band served as a useful positive control or marker in a number of experiments.

Might it be possible to prevent the differentiation of muscle cells or to destroy them by direct application of antimyosin antibodies? Singer's microinfusion method for prolonged injection of the regenerate was applied. The advantages of this procedure are that the test fluids bathe the growth directly without being diluted via the circulatory system (as with injection into blood vessels), and that it avoids possible barriers (as would occur with immersion treatments). Large quantities of normal serum affected several tissues of the regenerate adversely, but infusion of small quantities of muscle antiserum revealed sarcolysis attributable to the specific effects of the antibodies. A significant delay in the rate of regeneration was also noted in limbs infused with antiserum.

To demonstrate further the specific combining powers of the antibodies with muscle tissues, and in an effort to detect the site of localization of earliest synthesis of muscle proteins, regenerating limbs were treated with I^{131} -labeled antibodies by means of autoradiographic techniques. Grain counts of autoradiograms reveal a significantly higher localization of the labeled antibody in muscle cells than in other tissues, confirming the finding that the antibodies attach preferentially to muscle cells.

The demonstration of a nucleoprotein component in the regenerating limb raises a number of interesting questions. During regeneration an increase in this component precedes an increase in the quantities of other muscle proteins in the limb. After muscle has been formed, the amount of

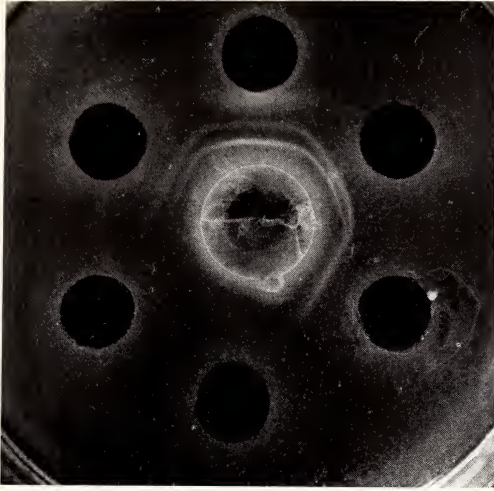


Fig. 3. Antigen-antibody precipitin reactions in agar. The central reservoir contains muscle extract. The peripheral reservoirs contain antisera produced by six different rabbits injected with purified muscle proteins. A minimum of three bands is seen in the reaction of muscle antigen with antiactomyosin serum, in the upper central part of the figure. To the upper right is the reaction of antimyosin serum with two components (actomyosin and myosin). Below it another antimyosin serum yields only one band of precipitate (myosin). In the upper left of the figure is an antiactomyosin serum with two components.

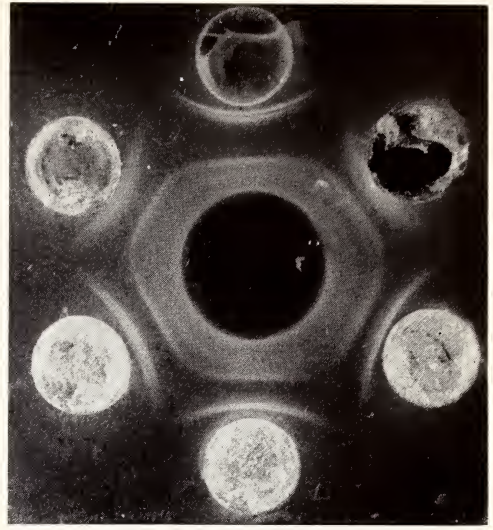


Fig. 4. Extracts of developing limb regenerates tested against antiserum to adult muscle antigens. In contrast to figure 3, the antiserum, which contains antibodies to actomyosin, myosin, and nucleoprotein, is in the central reservoir. Whole newt extract, tested in the top central reservoir, results in a minimum of three bands. To the right and reading clockwise, digital stages are tested, the first well containing the earliest stages and the least amount of muscle proteins, as progressively older stages are tested. The last reaction (upper left) is the same as its neighbor below except that the antigen was diluted. All these limb extracts contained muscle proteins.



Fig. 5. Hemorrhagic destruction of chick embryos at 12 (left and center) and 14 (right) days of incubation as a result of intracoelemic transplantation of adult spleen at 3½ to 4 days.

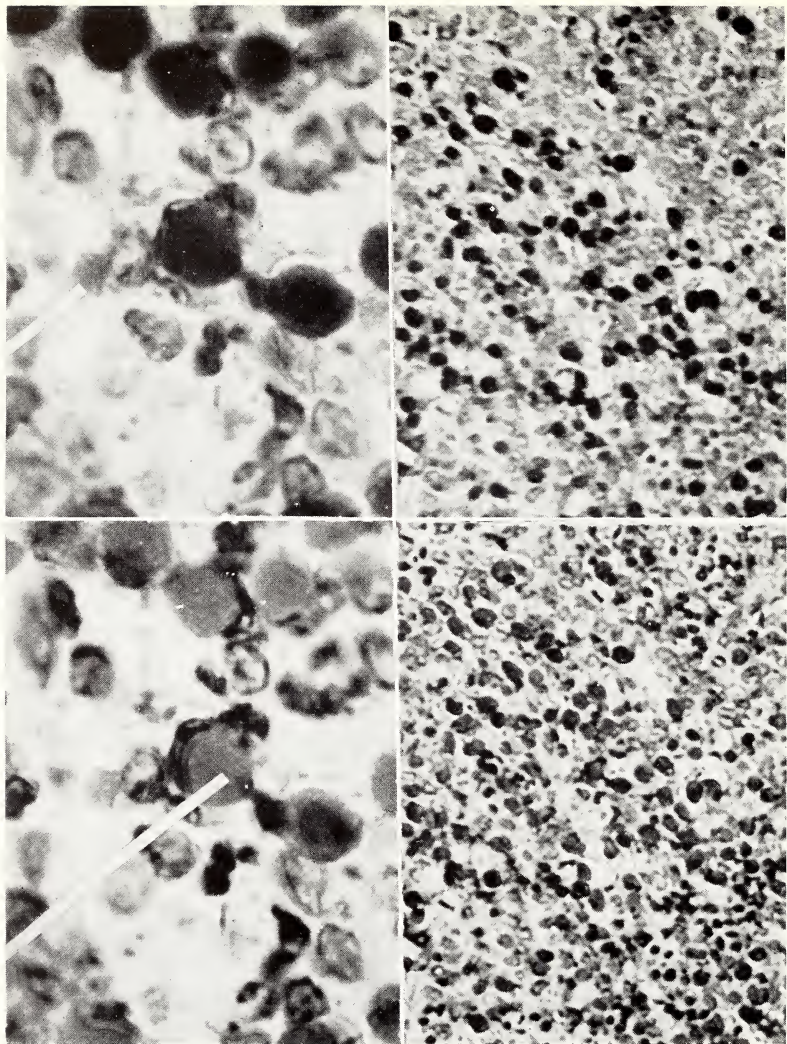


FIG. 6

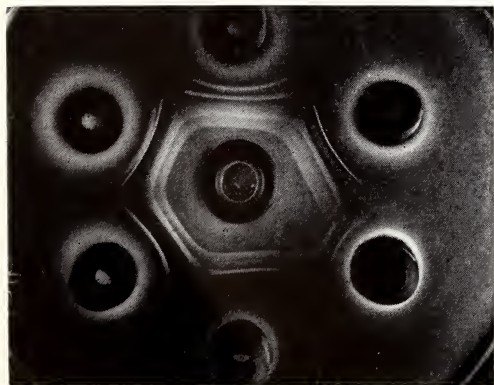


FIG. 7

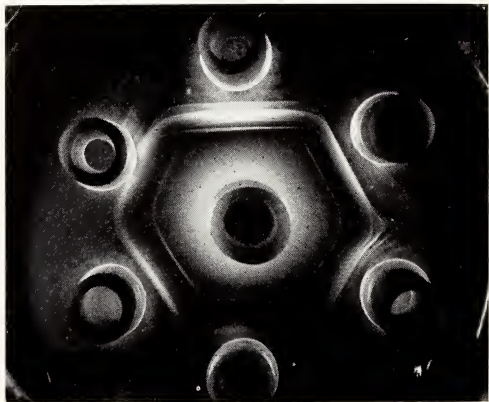


FIG. 8

nucleoprotein decreases. We may ask whether a direct relationship between nucleoprotein and specific muscle protein can be demonstrated in such a system. Experiments intended to answer this question are in progress.

One approach employs metamorphosed anurans, which have lost their ability to regenerate an amputated limb. The ability to regenerate an appendage gradually disappears in the frog tadpole at about the time of metamorphosis. In the past a number of experimental procedures have been found that stimulate this refractile system to regenerate. Singer has repeatedly emphasized the importance of the presence of intact nervous elements for the stimulation of regeneration. Indeed, in the absence of a sufficient quantity of nerves, regeneration does not occur even in the salamander, an animal which normally regenerates readily. Singer predicted and demonstrated that nonregenerating frogs can be stimulated to grow new limbs by augmentation of the nerve supply. More recently, he and his co-worker, Ashbaugh,

reported that nerve cells implanted into a denervated newt limb stimulated regeneration. Homogenates and extracts of nervous origin have so far been ineffective in producing the same result as intact cells.

What is the contribution of the nerves? To date, Singer has been able to rule out the "normal conduction function" of the nerve as a causative agent in initiation of regeneration. Only the quantity and not the quality or type of nerve seems to be important; moreover, ganglion implants without central connections promote regeneration. Therefore, the "acetylcholine mechanism" can be more or less relegated to a minor role. There are "neurosecretory" and "trophic" activities of nerves about which relatively little is known. Can the nerve be stimulating a basic mechanism of protein synthesis? Laufer believes that it is likely to be doing just this in some fashion. The first step in Laufer's approach, then, is to see whether the "incompetence" of nonregenerating limbs can be shown to be a biochemical lesion. If so, can it be alleviated with cellular ele-

Fig. 6. Four photomicrographs of the same area of a 4-micron section of the spleen of a guinea pig which had received a subcutaneous pellet of diethylstilbestrol 42 days previously. The tissue was fixed in neutral formaldehyde and stained with the Himes-Moriber triple stain for DNA, carbohydrate, and protein.

Upper left: 1000 \times before enlargement. Photographed with a green filter to emphasize carbohydrate. Dash terminates on an unusually large body of amorphous carbohydrate within a phagocyte. Part of the nucleus of the phagocyte can be seen between this amorphous carbohydrate and the larger, darker Foà-Kurloff body in the center of the photograph.

Lower left: 1000 \times before enlargement. Photographed with a red filter to emphasize DNA. Dash terminates on a Foà-Kurloff body. The nucleus of the Foà-Kurloff body cell can be seen as a cap on the left side of the body, just to the right of the nucleus of the phagocyte.

Upper right: 250 \times before enlargement. Photographed with a green filter to emphasize carbohydrate. Note the abundance of Foà-Kurloff bodies.

Lower right: 250 \times before enlargement. Photographed with a red filter to emphasize DNA. Note the absence of necrosis or lesion.

Fig. 7. An agar-diffusion plate containing an antiserum to insect blood in the center well and samples of insect blood in the peripheral reservoirs. The white lines in the agar between the reservoirs represent the precipitation of different protein antigens and their antibodies. The pattern for each reservoir differs from that of its neighbor because different concentrations and different sources of insect blood were used. When histochemical staining for esterase was applied to such an entire plate, only one band was clearly stained: in the top central reaction it was the second line from the top. The same component was colored in all the other antigen-antibody reactions as well. The comparable protein in neighboring reactions can be seen to become confluent with this band in a number of cases, confluence indicating probable identity.

Fig. 8. The central reservoir contains insect blood—a mixture of antigens. In the peripheral reservoirs have been placed antisera produced in five different rabbits after injection of blood antigens. The confluence of precipitate lines indicates probable identity of components. Note that, although the techniques and frequency of injection of the individual animals varied, the antibodies produced show a surprising similarity.

ments of defined composition? Since it is argued that nucleoproteins play a role in protein synthesis, can the nonregenerating frog limb be rendered "competent" by supplementing the supply of ribonucleoproteins or deoxyribonucleoproteins already present in the tissues? Experiments intended to answer these questions are being carried out by Dr. Laufer in collaboration with S. J. Cantolino, a student in the Johns Hopkins University School of Medicine. Further complementary and parallel experiments are being conducted in collaboration with Dr. Singer.

Studies on the Development of Avian Hemoglobin

Robert G. Beard's efforts to obtain precipitating antibodies in rabbits against purified chicken hemoglobin were successful in that 8 animals out of 17 injected gave antisera with titers (in terms of dilution of a 10 per cent hemoglobin solution) ranging from 1/6400 to 1/25,600.

The hemoglobin preparation used for injections was in the carbon monoxide form. The preparation was purified as reported in Year Book 56, and concentrated by lyophilization to give a stock solution of approximately 10 per cent avian carbonylhemoglobin in distilled water. It was Seitz-filtered and stored frozen in sterile vials, each vial containing enough antigen for one day's injections.

Two methods of immunization were employed: multiple intravenous injections of relatively large amounts of the stock antigen, and a single massive injection of hemoglobin combined with the complete Freund adjuvant. Rabbits receiving the adjuvant mixture were given 150 mg of hemoglobin in adjuvant injected subcutaneously in the nuchal region. They were bled 9 weeks later at the same time the intravenous series was bled. Each of the rabbits receiving intravenous injections was given three 100-mg injections per week of hemoglobin, the antigen being administered both intraperitoneally and intra-

venously. These injections were continued for a period of 7 weeks, the rabbits being bled 2 weeks after the last injection. Of the 14 animals that survived for the duration of the experiment, 8 produced antibodies in significant quantities. Of the 8, only 3 had received the hemoglobin-adjuvant mixture, the remainder having been injected with hemoglobin alone. All the animals that failed to respond had been treated with the antigen-adjuvant combination.

The animals receiving the adjuvant reacted poorly, contrary to general expectations, whereas the animals receiving long-term injections of large amounts of antigen reacted uniformly well. The question remains whether the response of the latter group might not be due to trace amounts of impurities in the antigen preparation rather than to the hemoglobin itself.

The antisera were assayed by means of the interfacial ring test. They were found not to cross-react with fat-free yolk extracts; they reacted weakly with a saline extract of fresh egg white, as did the control sera; and they reacted well with chicken serum which in all probability contained traces of hemoglobin resulting from slight lysis of the red blood cells during the collection and isolation of the serum.

Previous examination of the antigen by means of a continuous-flow paper electrophoresis apparatus had indicated the existence of two hemoglobin components differing slightly in electrophoretic mobility. The use of the starch gel zone electrophoresis method of Smithies (veronal buffer, pH 8.6, ionic strength 0.05; 7 milliamp/strip for 12 or 24 hours at 2° C) gave a good separation of the two hemoglobins. The minor, more rapidly migrating component represented 10 to 15 per cent of the mixture. This finding is in good accord with the results of Huisman and Van der Helm, who were able to isolate and analyze the two chicken hemoglobin components by means of column chromatog-

raphy on ion-exchange resins. Normal chicken serum components were not detected in the antigen by the starch gel method. The absorption spectrum of the antigen was in accord with reported values and showed no peak in the ultraviolet characteristic of nucleic acids, indicating a clean separation of hemoglobin from nuclear components.

One of the antisera was tested by double diffusion methods in agar in capillary tubes in an effort to determine the minimal number of reacting groups in the system. Serum was overlain by 1 per cent agar in 0.9 per cent NaCl, which was in turn overlain by 0.1 per cent hemoglobin. After 4 days, three bands were visible in the agar: a heavy band near the serum-agar interface, and two lighter but sharper bands close together in the middle of the agar column. These bands intensified with time, and no new bands were seen to ap-

pear, indicating a minimum of three antigens in the system, but which, if any, of them represents one or even both of the hemoglobin components remains to be determined. Absorption studies designed to test this proposition are under way at present.

In a preliminary study, six of the antisera were tested against extracts of chick embryos to determine the first appearance in the embryo of reactive groups common to the antigen. Embryos of Hamburger and Hamilton stages 4 through 13 were tested. The control sera gave no reaction, but five of the antisera tested gave positive reactions with extracts from all stages. The one remaining antiserum gave a positive reaction only from stage 8 onward. These experiments must be repeated along with more extensive absorption studies before such preliminary results can be interpreted.

THE ACQUISITION OF BIOLOGICAL SPECIFICITY

In an analytical article, "The acquisition of biological specificity," completed during the year for publication in *The Cell*, Dr. Ebert attempted to evaluate critically the response of developing systems to implanted adult tissues. It is a fundamental premise that for a substance to be antigenic it must be foreign to the test animal. Burnet and Fenner were the first to postulate that each cell acquires during its development a "self-marker" component, which prevents the organism from making an immune response to constituents of its own cells. But what of the "exceptions to the rule"? If autologous or homologous tissues are modified slightly they may stimulate an immune response. Moreover, although the alteration has been sufficiently great to make the inoculum antigenic, the antigens are still closely related to the proteins of the host, so closely, indeed, that the immune reaction elicited may not distinguish the altered from the normal form. As a consequence the normal host tissue may be destroyed.

Thus it has been shown that the injection of normal monkey nervous tissue combined with adjuvant into monkeys results in encephalomyelitis. Under some circumstances antigens of an individual may call forth in the same individual the production of antibodies, or elicit a cellular response without artificial intervention. The process is called autoimmunization, and it is thought that a number of serious diseases may be caused in this way (e.g., acquired hemolytic anemia, rheumatic fever, rheumatoid arthritis, glomerulonephritis, sympathetic ophthalmia). Almost without exception the process that enhances the antigenicity of the patient's own tissues is obscure.

Autoimmunization is an important basic problem; our immediate concern is with the modification of development by the exposure of a developing organism to homologous adult tissues. It can be said without fear of contradiction that this simple phrase "exposure of a developing organism to homologous adult tissues" has

profound implications for the student of development, for it marks the focal point at which, within the past five years, three major research trails have begun to converge. The background and motivation of these investigations were presented briefly in Year Book 56 (pp. 322-336) and in the fully documented article "The acquisition of biological specificity."

During the year covered by this report, important progress has been made in several aspects of these related problems. Although a full re-examination of the objectives of the investigations is beyond the scope of this report, the following general statement may serve as a guide. Tissue transplants, whether made to the adult or to the embryo, emit complex matter, including viable cells, intracellular particulates, and large molecules that operate in regulating, either qualitatively or quantitatively, or both, the synthesis of macromolecules in the host. The modification of the patterns of synthesis in the host may be achieved directly as a consequence of the predominant localization of the graft cells or molecules in the homologous organ of the host or indirectly by the localization in that organ of antibodies or products of immune reactions directed against the antigens in the original inoculum, or by a combination of these events. In all these phenomena we find recurring one fundamental theme, namely the possibility of the constant emission by normal cells of specific regulatory substances, a physiological transaction still relatively obscure.

Induced Aspermatogenesis

The cytological and functional degeneration of the guinea pig testis that results from the injection of homologous testicular tissue combined with adjuvant was described in Year Book 56. During the year covered by this report, many of the findings summarized previously were documented in papers published by Dr. Seymour Katsh in the *Journal of Experimental Medicine* and *Nature* and by Dr.

Katsh and Dr. D. W. Bishop in the *Journal of Embryology and Experimental Morphology*. It will be recalled that a study of the male rabbit and rat indicated that induced testicular destruction is not limited to the guinea pig. Moreover, it was shown in a parallel investigation of the female guinea pig that, although the ovary is insensitive to induced gonadal injury, the capacity to bear young is reduced, possibly as a result of an anaphylactoid response involving the uterus of the injected female animals. In both sexes and in all the species studied the response to the administration of gonadal tissue homogenate and adjuvant indicates that an immune reaction is involved. The mechanism of the reaction is not well understood. Therefore work has been continued actively along several lines.

Chemical nature of the antigen. Dr. Katsh has continued studies aimed at the chemical identification of the antigen present in the testis which, when combined with adjuvant, can induce aspermatogenesis. The antigenic component begins to appear in quantity in the testes of guinea pigs between the first and second months after birth, at a time during spermiogenesis when secondary spermatocytes are being transformed into mature sperm. Antigenicity is associated with intensely staining polysaccharide granules in or near the acrosome. Katsh has noted that the sperm of certain forms, including the guinea pig and man, possess acid-fast characteristics like some of the mycobacteria. The sperm of other forms, e.g. opossum, rabbit, rat, and rooster, show little, if any, acid-fast stainability. This observation may be important in explaining the relative ease with which aspermatogenesis can be induced in some forms and not in others. It is considered likely that acid-fastness is related to the antigenicity of the sperm involved in the induction of aspermatogenesis.

Contributions of the bacterial component of adjuvant. Adjuvant without bacteria (incomplete adjuvant) is incapable of me-

diating testicular destruction. It is believed, therefore, that the bacterial component participates in the reaction. The bacteria employed in adjuvant to induce aspermatogenesis are acid-fast organisms (*Mycobacterium tuberculosis* or *Mycobacterium butyricum*), suggesting that acid-fastness is related to the ability of the bacteria to act as mediators of aspermatogenesis. If the acid-fast material is an essential attribute of the bacterial component, its removal should render bacteria ineffective. Also, it should be possible to isolate and identify the effective substance. Male guinea pigs were injected with a variety of bacteria (acid-fast, nonacid-fast, and acid-fast bacteria that had been rendered nonacid-fast) in adjuvant. Other groups of guinea pigs were injected with antigen plus adjuvant containing a variety of chemical components extracted from acid-fast and nonacid-fast bacteria. It has been shown that: (1) acid-fast bacteria are more potent than nonacid-fast bacteria in mediating aspermatogenesis; (2) tubercle bacilli that have been rendered nonacid-fast are incapable of mediating testicular damage; (3) the specific chemical factor from bacteria that can replace the bacteria in adjuvant is a lipopolysaccharide; lipopolysaccharide from *Mycobacterium tuberculosis* is more potent than that obtained from nonacid-fast bacteria.

Smooth muscle contraction during anaphylaxis. In the course of Dr. Katsh's analysis of the anaphylactoid response of the sensitized uterus to sperm, other organs containing smooth muscle, including ileum, vas deferens, and seminal vesicle, were tested for their response to the specific antigen, homologous sperm, *in vitro*. Seminal vesicles and vasa deferentia did not respond, but ilea of the animals with testicular damage always contracted when tested in this manner; none of the ileal segments from animals without testicular lesions responded. Other experiments demonstrating that the aspermatogenic condition is reversible with time also revealed that, when spermatogenic ability is recov-

ered, the ileal segments no longer react *in vitro*.

These results raise several interesting questions. For example, why do the seminal vesicles and vasa deferentia not respond in the test for anaphylaxis if, as is commonly stated, all organs containing smooth muscle contract upon presentation of the specific antigen to a sensitized animal? What significance can be attached to the fact that only ilea of animals that had testicular lesions were responsive to the specific antigen?

In attempting to answer these questions, several experiments have been performed to lead to a working hypothesis correlating the anaphylactic response of the ilea with the aspermatogenic condition. The hypothesis, briefly stated below, is being tested for its applicability to the general problems of smooth muscle contraction during anaphylaxis.

The intracutaneous injection of the antigen-bacteria-oil mixture results in a depot of these materials in the skin, where they are incubated at body temperature. During the incubation period, the oil elicits a cellular response, i.e. infiltration of the injection site by reticuloendothelial cells and cells of the antibody series such as lymphocytes and plasma cells. The oil also serves as an extractive medium in which the specific components of the bacteria are leached out of the bacterial cells. The lipopolysaccharides, which are now in the oily phase, combine with the antigen to form an antigen-hapten complex. The complex is transported through the body by macrophages and circulating cells. In organs containing cells of the antibody series, antibody synthesis is stimulated by the antigen brought to them. In such organs as the ileum, the presence of large amounts of lymphoid tissue connotes a large capacity to form antibody. Thus, the isolated, sensitized ileum, when being challenged with antigen, responds by contracting, owing to the release of some unknown factor produced during antigen-antibody interaction. The magnitude of

the response is related to the number of antigen-antibody interactions.

The events that occur in the testis, according to this view, are the following: The antigen-hapten complex is brought to the testis and interacts with the cells of the antibody series in that organ and in the lymph nodes. A toxic factor released during the interaction induces the cytolysis of spermatogenic cells, resulting in the release of more antigen. The further release of antigen provides more antigen units to react with cells of the antibody series. Thus the process of spermatogenic tissue destruction can go to completion because of this self-generating interaction of antigen with antibody-containing cells.

In organs containing no cells of the antibody series, provocation with antigen is without effect because there is no interaction of antigen with antibody. For this reason the seminal vesicle and vas deferens of the exsanguinated animal do not respond to antigen *in vitro*. The idea may explain how the isoallergic diseases can occur spontaneously: as a result of infection and damage to an antigenic organ, a factor from the infectious agent forms a hapten complex with the "native" antigen; thus, antibody is formed against the organism's own organ(s).

The immune mechanism. Although sperm-agglutinating and sperm-immobilizing antibodies appear in the sera of animals injected with sperm or testicular extract plus adjuvant, there are sound reasons (presented in Year Book 56) for believing that these circulating antibodies are not instrumental in inducing aspermatogenesis. To cite only one argument, aspermatogenic activity cannot be transferred passively by the transfusion of serum of treated animals. We may inquire, however, whether aspermatogenesis is related to the reaction by which a homograft is destroyed, and whether transference of immunity might be achieved by a massive transfer of cells from lymph nodes draining the site of injection, i.e. by what Medawer has called adoptively acquired immunity. The lymph

nodes of adult animals injected with testicular homogenate have been removed and implanted into secondary male hosts. Although the nodes have persisted, there has been little testicular damage. In other experiments, Katsh has tried to induce aspermatogenesis by transferring suspensions of lymphoid cells from sensitized guinea pigs to uninjected males. The results have been inconsistent, but of sufficient promise to warrant further exploration.

Evidence of the nature of the phenomenon may be sought in another way. If an immune reaction is involved, it should increase with the age of the recipient animal. In the newborn animal, immune reactions are absent or negligible, serological maturity being reached several weeks or months after birth. Dr. Bishop has found that the injection of adult testicular homogenate with adjuvant into newborn guinea pigs, 1 to 10 days of age, fails to induce aspermatogenesis. It should be emphasized, however, that the failure may be due either to the lack of an antibody-forming mechanism or to the undeveloped condition of the infantile gonad.

Dr. Ebert's recent findings suggest that the first alternative can be tested by introducing adult antibody-forming splenic or lymph node tissue into the newborn animals at the time of injection of testicular homogenate. As Dr. Ebert has emphasized, however, not only may there be species differences in the ability of adult tissue to carry on immune reactions in neonatal animals, but also, within a single species, the ability to respond to different antigens may mature at different times. In addition, the work by Wytenbach and others raises the question whether the injection of testicular homogenate plus adjuvant into neonatal guinea pigs might induce tolerance, so that the animals would not only fail to show signs of aspermatogenesis on the first injection but would fail to react on subsequent challenge. The guinea pig is among those species that mature early, at least in many respects, and it might be

expected that tolerance would not be bestowed by an injection after birth. Preliminary results indicate that this expectation has been realized.

Acquired Tolerance to Organ Extracts

Mr. Charles R. Wytenbach, Predoctoral Fellow of the National Science Foundation, has begun to probe more deeply into the problem of tissue specificity, using immunochemical techniques. At the start of his program the principal target was the nervous system. To what extent is it possible to characterize the specific proteins of the distinctive morphological regions of the central nervous system? It was clear that, in order to obtain immunochemical resolution, highly specific antibodies to cerebrum, mesencephalon, and cerebellum had to be developed. Conventional methods have failed to provide the precise tools needed for the study. Precipitin and absorption reactions and Ouchterlony agar-diffusion tests indicate identity or close similarity in the antigens of the three morphological regions. Antibrain antibodies are evoked by the injection of homogenates of adult chicken brain into rabbits, as had been expected after Dr. Ebert's earlier success with a similar approach. Despite variations in the method of preparing antigens, injection schedules, and titration techniques, however, the highest antigen dilution to give a precipitate with a 1 to 5 dilution of serum was 1/2560.

The most effective antisera were absorbed with chicken red blood cells and compared with the corresponding unabsorbed sera with respect to cross reactivity with liver, muscle, and spleen. All the absorbed sera were less effective than the unabsorbed sera, regardless of the antigen used, the difference varying from one to three dilutions. There was no correlation of the extent of loss of activity with either the antigen or the serum used. As an example, let us consider one antiserum against cerebrum. The same amount of

precipitate was obtained with the following concentrations (in gammas per milliliter) of antigen: cerebrum 30; liver 75; spleen 90; and muscle 190. Hence the cross reactivity was considerable. Plans to absorb these sera with liver, muscle, and spleen were abandoned, inasmuch as a more promising approach was found to be available.

The failure to obtain antisera highly specific to distinct regions of the chicken brain prompted Wytenbach to adopt a different approach, based on a brief report by Feldman and Yaffe, who described the successful induction of immunological tolerance by exposing newborn rabbits to small quantities of the organ extracts. Their findings indicated that, if newborn rabbits are subjected to homogenates of mouse heart, upon subsequent challenge in later life with mouse brain, a specific antiserum to brain is evoked which will not cross-react with heart. Wytenbach reasoned that, if newborn rabbits were injected with a number of nonnervous chicken tissues, then, on subsequent challenge with brain, an antibrain serum with high specificity should be developed.

Liver, spleen, and muscle (LSM) in equal quantities (on the basis of nitrogen determinations) were selected for the initial injections. In addition a group of newborn rabbits was injected with mesencephalon, for, if immunological differences exist between different parts of the central nervous system, it might be possible to demonstrate them by making rabbits tolerant to antigens of one region and then challenging them with an antigen of another. Feldman and Yaffe's report was not documented fully; since their results were variable, one may ask how significant they are. The total amount of heart injected starting at birth was only 36 mg, given over a 2-month period; therefore probably only 25 per cent of the antigen was injected during the immature period. How small a dose of antigen is sufficient to produce tolerance? Is it an all-or-none

phenomenon? If partial tolerance is obtained (as revealed by precipitin tests), is it a true partial tolerance of each antigen, or, with the heterogeneous mixture of antigens present in an organ extract, is it in fact a complete tolerance to certain antigens and a lack of tolerance to others? Is tolerance permanent or temporary? In short, the phenomenon of induced tolerance to organ extracts is of interest in itself.

As Wyttenbach's work progressed, it became increasingly clear that any success in obtaining specific antibodies to component parts of the nervous system would hinge on a better understanding of tolerance to tissue extracts. With the encouragement of the Director, Mr. Wyttenbach has begun a large-scale, long-range study of the acquired tolerance reaction. The details of the study obviously cannot be given here. It will suffice to point out that Wyttenbach has been able to induce tolerance to liver and spleen antigens. Although these findings must be regarded as tentative, pending verification, they offer exciting possibilities. In contrast, the first results in attempts to induce tolerance to mesencephalon have been less gratifying.

The scope of the program is indicated by the fact that, in addition to numerous smaller pilot experiments, two major experiments involving about 100 newborn rabbits each are in progress. For example, of one group of 94 newborn rabbits, the following experimental groups were established: 15 received a total of 100 mg of antigen equally divided among chick liver, spleen, and skeletal muscle (LSM); 12 received a total of 50 mg of LSM; 12 received a total of 50 mg of chick mesencephalon; 11 received either 2 or 4 mg of mesencephalon with adjuvant; two groups of 11 each were given 50 mg and 25 mg of chick liver; 22 were divided into three groups receiving 100 mg, 50 mg, or 25 mg of chick spleen. Only a few technical details should be mentioned to enable the reader to appreciate the nature of the research.

Mesencephalon was prepared by homogenizing the tissue in the ratio of 1 g of mesencephalon to 2 ml of chick Ringer's solution. The rabbits received the whole homogenate less the larger debris that would not pass a 24-gauge needle. Liver was homogenized in the ratio of 1 g tissue to 3.5 ml of saline and centrifuged for 10 minutes at 2800 rpm. Spleen was homogenized in the ratio of 1 g of tissue to 3 ml saline, followed by centrifugation at 1800 rpm for 10 minutes. Muscle was homogenized in the ratio of 1 g of tissue to 2 ml saline and centrifuged at 1000 rpm for 10 minutes. Of the LSM preparations only the supernatant was injected. A Ten Brock homogenizer was used on all tissues. Protein determinations were based on Nessler's colorimetric test for nitrogen.

Nearly all the rabbits received the total dose in 9 daily injections beginning on the day of birth. A few received it spread over 7 or 10 days. All injections were intraperitoneal except those with adjuvant, which were subcutaneous. Weights were recorded daily during the injection period so that, if necessary, a dose/weight ratio could be determined. Although there is no evidence that the tissue homogenates are toxic to newborn animals, losses were heavy, owing chiefly to the loss of entire litters abandoned by the mother. Thus, of 94 rabbits treated in this first experiment, only 55 survived to 8 to 10 weeks, regarded by some investigators as the onset of the period of serological maturity. Despite these and other obstacles, the results in the liver and spleen series are remarkably consistent.

To determine the experimental procedure for the challenging doses, a preliminary experiment was carried out in which several adult rabbits were injected by various routes and with different doses of spleen. It revealed that a dose of 75 mg/kg in 5 injections on alternate days gives as high a titer as a total dose of 100 mg/kg. Bleedings at 5, 7, and 9 days after the last injection revealed that peak titer

is reached no earlier when the last injection is intravenous than when it is mostly intraperitoneal. Titers with sera obtained on the fifth, seventh, and ninth days were essentially the same. Hence it was decided to give 5 injections on alternate days and to inject the liver and spleen groups with a 75 mg/kg total dose. Rabbits challenged with cerebrum or cerebellum received a total of 125 mg/kg because of the poor antigenicity of these preparations. Portions of both the fourth and fifth injections were given intraperitoneally to reduce the chance of emboli from large intravenous doses.

Let us consider briefly the observations on the spleen and liver groups. All bleedings were done at 5, 7, and 10 days after the last injection except for part of the spleen group in which 9 rabbits were bled on days 7, 10, and 13. The 13-day bleeding was designed to detect any delayed peaks. Invariably, however, the 7- and 10-day bleedings gave titers greater than those at 13 days. Therefore the 13-day bleeding was replaced by a 5-day bleeding. In nearly all rabbits bled on the 5-, 7-, and 10-day schedule the 5-day titer was higher by one dilution than the 7- and 10-day titers.

In the "spleen group," positive controls, i.e. previously untreated, serologically mature animals challenged with spleen homogenates, gave titers of 1/5000. Of 14 test animals injected with spleen homogenates at birth, 7 were found to be completely tolerant, 5 showed partial tolerance, and only 2 showed no tolerance at all upon challenge at maturity. Liver appeared to be less antigenic, as the positive controls gave titers of 1/2200 and 1/1100. In the "liver group" 50 per cent of the treated rabbits were fully tolerant; of the remainder none showed a full immune response.

Rabbits may weigh from 35 to 75 g at birth. Hence within each subgroup an attempt was made to find a correlation between weight at birth and the degree of

tolerance. There is a slight indication that this factor must be considered. Yet the fact that it is slight points up the variability in the system. This variability is demonstrated emphatically by the fact that, whereas 25 mg of antigen is sufficient to produce complete tolerance in some rabbits, 100 mg is not sufficient in others.

An Immune Reaction: "Graft-against-the Host"

In studies of the destruction of kidney homografts some five years ago, Dempster and Simonsen posed the question whether a graft containing cellular elements normally active in immune processes might react against its host, a possibility overlooked by others who were focusing attention on the more "conventional" host-versus-graft reactions. The "graft-against-the-host reaction" has become an important link in our understanding of graft-host relations as a result of a series of recent independent findings in which cells and tissues involved in immune processes have been implanted into hosts incapable of an immune reaction.

Jacobson's discovery that the lethal effects of whole-body irradiation could be prevented by shielding the animal's spleen led to a series of noteworthy discoveries. Jacobson proposed several possible explanations for the spleen-shielding experiments, including humoral stimulation by a subcellular agent, and colonization of damaged areas by cells of the shielded tissue. After the demonstration that several species of animals subjected to a lethal dose of irradiation are kept alive by intravenous injection of a normal bone marrow cell suspension after the exposure, it was established that blood-forming tissues are specific in causing recovery; hemopoietic cells are required. It has been shown convincingly that, when the hemopoietic tissues of the host have been destroyed, "reseeding" by blood-forming cells is a requirement for recovery. It is a question of some controversy whether re-

covery can be obtained with cell-free extracts of hemopoietic tissues. Although the evidence strongly favors the involvement of cells, the possibility that cell-free extracts may be effective when the host's blood-forming tissues are not completely destroyed cannot be discounted altogether. The proof that bone marrow and peripheral blood cells in the animals receiving bone marrow injections are derived from donor cells was obtained by a variety of techniques (cytochemical, cytological, and immunochemical), applying the basic principle that homologous or heterologous donor cells can be identified by virtue of differences between them and the corresponding host cells. Perhaps the most convincing demonstration is that of Ford and his colleagues, who showed that dividing cells in bone marrow and lymph nodes of surviving host mice had chromosomes cytologically resembling the homologous donor cells which carried a chromosome marker.

It was soon found, however, that, when homologous or heterologous animals are employed as donors (rather than isologous animals, animals of the same inbred strain), recovery and survival for 30 days is followed by the death of many of the hosts. The response is characteristic; the lymph nodes and to some extent the spleen go through a granulomatous reaction, resulting in fibrosis and fibrinoid necrosis, a delayed reaction interpreted by many investigators as an immune reaction, in which the host tissues are affected by immune reactions of the donor cells.

In contrast, Makinodan and others believe this explanation to be an oversimplification and, in fact, favor the view that death is due to a recovery of the radio-sensitive antibody-producing cells of the host, which now react against the proliferating foreign bone marrow. The possibility that both reactions may occur is also recognized. In irradiated mice injected with rat bone marrow, rat serum proteins cannot be detected; if present, according

to Makinodan, their concentration is lower than 12 μg of protein per milliliter of serum. Makinodan suggests that this finding indicates that the graft is not producing antibodies against the host. Although his argument holds only for circulating antibody, it may be valid, for in heterologous combinations circulating antibodies usually can be detected. When normal isologous nucleated bone marrow cells were injected into irradiated mice along with rat red blood cells, circulating agglutinins against the rat cells are not detected, indicating inability of the bone marrow cells to produce antibody.

This finding is the focal point of some controversy. The time of the delayed bone marrow reaction and the severity of the reaction are dependent on the dosage of irradiation (faster recovery of immune reactions following lower dosage, leading to a more severe reaction). If antibody-producing cells are derived from the transplant, the death rate should increase with an increase in bone marrow dose. In contrast, Makinodan has dealt largely with heterologous combinations in which the host reaction seems to predominate. In homologous combinations, the graft reaction appears to take precedence, suggesting that the ability of antibody-producing cells to recognize "foreignness" may be measurable.

As was stated in Year Book 56, independent discoveries in several laboratories of deleterious effects of cell suspensions and splenic grafts were interpreted as graft-versus-host reactions. In the study by Billingham and Brent a number of mice of strain A made tolerant by injecting adult CBA spleen cells died within 2 to 3 months after birth. Characteristically, the lymphoid tissues of these animals were grossly aberrant: the lymph nodes were lacking, the spleens fibrotic and deficient in Malpighian corpuscles; even Peyer's patches were missing.

Simonsen demonstrated deleterious effects in the host spleen after the intrave-

nous injection of leucocytes or spleen cells of juvenile or adult chickens into the 18-day chick embryo. His conclusion that splenic enlargement and subsequent damage are due to colonization by donor cells appears sound. He has been able to propagate the cells through nine consecutive passages without significant loss of activity. These two findings seem to be conclusive. He states that the recipients have to be of an age at which tolerance of homologous cells can be induced and that donors must be old enough to form antibodies. According to him, preliminary evidence in support of this hypothesis can be seen in experiments in which 11-day or 18-day embryos of White Leghorn stock were inoculated intravenously with spleen cells of 18-day Brown Leghorn embryos. The group injected on the eleventh day comprised only 5 animals. When sacrificed at 3 days after hatching they showed no anatomical changes. The group injected on the eighteenth embryonic day contained 31 animals, 14 of which were likewise sacrificed at 3 or 4 days after hatching and found to be normal. Transplantation of embryonic chicken spleen cells was ineffective. Curiously enough, Simonsen also found that leucocytes of other avian species, pigeon and turkey, did not cause splenic damage in the chick. It is difficult to reconcile this observation with the graft-against-the-host hypothesis. Chorioallantoic grafts of adult spleen result in the growth of the homologous organ; as DeLanney and Ebert discovered, however, when grafts are allowed to continue beyond the seventeenth day, a small but consistent fraction of the host spleens undergo a striking degenerative change characterized by the destruction or modification of the vascular bed, leading to a stasis in flow of granulocytes, their accumulation in cystic masses in the spleen, and eventual death. The cystic condition of the spleen occurs with a frequency approximating that of the death of chicks at or shortly after hatching. DeLanney

extended these observations to the salamander, *Taricha torosa*. Grafts of adult salamander spleen made either into the coelom or into a pocket in the dorsal fin of the larval salamander evoke not growth stimulation, but suppression of the host spleen.

The importance of these observations is beyond question. They afford a further demonstration of graft-host interaction, clearly suggesting the passage of host substances capable of an antigenic stimulus, thus lending further support to the idea of a continuous circulation of specific products in the embryo. But the evidence that the immunologic response comes from the graft has been far from complete. That the reactions in the recipient animals occurred late in embryonic life or after birth left open the possibility that the phenomenon might be a type of autoimmunization reaction. Thus it was essential to demonstrate that *the host can be destroyed before its own mechanisms for an immunologic response have developed*.

During the year this goal has been achieved by Dr. Ebert working with W. D. McCleary. The experiments were an extension of those initiated by Dr. Ebert in collaboration with Miss C. M. Coffman, in which grafts of adult chicken spleen were made to the coelom of the 4-day chick embryo. Initially grafts were made to 4-day embryos, which were permitted to develop until the tenth day, when the host spleens were harvested and used as the donor tissue for a second set of grafts, and so on, the experiment being terminated after the fourth set of grafts was recovered. On the average the host spleens were more than five times larger than appropriate controls.

As in earlier studies employing the chorioallantoic technique, histological analysis of the host spleens argues against the transfer of large populations of adult cells; in fact, the primitive architecture of the host spleen in the period covered by the experiment makes it unlikely that adult

cells would not be detected. The transfer of a seed population of graft cells is not ruled out, however. In fact, since the growth-promoting activity is not diluted by serial passage, but is maintained relatively constant, the most economical explanation of the observations is the colonization of the host spleen by whole cells from the donor, cells capable of reproduction.

When grafts are allowed to persist until the twelfth to fourteenth day of incubation, i.e. from 8 to 10 days after transplantation, a strikingly different picture emerges. In more than 30 per cent of 244 successful operations, there is a profound destruction of the entire vascular bed of the host embryo, resulting in hemorrhagic lesions like those pictured in figure 5, plate 1. In other embryos the effect is less pronounced. In this type of experiment an immunologic reaction on the part of the host appears to be eliminated, for the cells generally believed to be responsible for immune reactions are lacking at the time the effect is best observed. It may be mentioned, with the permission of Dr. L. E. DeLanney, that recently he has been able to achieve a similar result by implanting a "rod" of adult salamander spleen into a "tunnel" in the somite region of salamander embryos at stages 25-28.

Thus attention is focused on the immunologic capacity of adult grafts in an embryonic environment. The evidence strongly argues for the capacity of such grafts to exert effects of a class usually described (for want of more precise information) as cellular immune mechanisms. We may ask whether such grafts will also produce circulating antibodies of the more conventional type. Fundamental to this possibility is the postulate that adult tissue can produce antibodies when it is presented with the antigen for the first time in an embryonic environment. There is no agreement on this question. Some investigators have stated that antibodies are not formed; others believe they have

demonstrated antibody production under these experimental conditions.

In an effort to clarify this problem, Mr. John Goffinet has begun to utilize the well studied systems of chorioallantoic transplantation of spleen, combined with intravenous administration of the antigen bovine serum albumin. Although fragmentary results indicate that adult spleen can produce antibodies in an embryonic environment, the findings must be regarded as tentative.

Organ-Specific Coordination of Differentiation and Growth

In view of the ultimate destruction of the host tissues, Dr. Ebert has questioned the advisability of using lymphoid organs like spleen for further tests of organ-specific regulation of differentiation and growth. Although organ-specific agents other than those involved in the immune response appear to be involved, the overwhelming power of the immune mechanism may render the description of other systems difficult. Simonsen believes the graft-against-the-host reaction to be the single mechanism involved. The evidence does not permit such a conclusion. Neither experiments using as donors and hosts members of a highly inbred line of chickens (in which incompatibility should not be manifested) nor experiments employing filters which preclude cellular transfer from graft to host have been fully convincing. In the latter experiments, initiated by Dr. Ebert in collaboration with Dr. Jacques Mulnard, splenic grafts encased in millipore filters evoked a significant increase in the size of the homologous organ of the host. The increase was of the order of 30 per cent, however, far less than the 100 to 500 per cent attained by grafts in the absence of any filter; moreover, in histological studies, grafts within the filter were found not to have the full spectrum of cell types which characterize the normal graft.

Efforts are being continued to obtain

positive evidence of a growth-stimulating effect of noncellular organ preparations, such as has been described by others. It is clear that such organ preparations exhibit properties enabling them to localize predominantly in the homologous tissue. Evidence has been cited of the efficacy of frozen, thawed, and other nonviable preparations, yet most of it falls short of being wholly convincing. Although criticism of the use of lymphoid tissues appears to be justified, criticism of the hypothesis as a whole is unwarranted.

In continuing this aspect of the program, progress can be reported only on a technical level. John A. Goffinet has mastered a technique, derived from that of Terasaki, for the intravenous injection of the 9-day chick embryo. It involves placing the shaft (the lock having been removed) of a 30-gauge needle in one of the large chorio-allantoic veins and anchoring the needle in position with paraffin. A stylet is placed in the lumen of the needle after the injection, and the needle is left in place until about the sixteenth day of incubation. This method permits daily intravenous injections to the same embryo, a feature of obvious experimental value. The mortality in the week following the injection has been found to be less than 10 per cent in a series of over 100 embryos. Of interest is the finding that, if the needle is withdrawn from the vein after injection of a 9-day embryo, approximately two-thirds of the embryos die from failure of vasoconstriction and consequent hemorrhage. The needle must therefore be left in the vein. If the injection is made into a 10-day embryo, however, the needle can be withdrawn from the vein with little or no resulting hemorrhage and mortality.

Goffinet has begun to use the technique to study the effectiveness of injecting cell-free homogenates, centering his attention on the heart. The toxicity of the homogenates has so far proved to be a formidable obstacle. As little as 2 mg of adult or embryonic heart, in the form of homogenate,

kills almost instantaneously every embryo injected. The toxicity cannot be explained on the basis of bacterial contamination, or by contamination by any particle reproducing *in vitro* (after 48 hours the homogenate is somewhat less toxic), or by potassium toxicity, or by hypervolemia. After centrifugation of the homogenate at 8700g for 10 minutes, the supernate is devoid of toxic effect, thus showing that a small molecule is not responsible. At present the most likely explanation for the toxicity of homogenates is a sudden shower of small emboli probably formed after the introduction of the injected material into the blood stream. Goffinet has shown that multiple small emboli can indeed cause virtually instant death of the 9-day chick embryo. He has found that injection of approximately 105 *Lycopodium* spores, inert spheres of remarkable uniformity of diameter (ca. 30 μ), also results in instant death of the embryo.

Goffinet has also begun experiments exploring the growth-stimulating effect of organs not noted for their antibody-synthesizing capacities. He has taken the heart as his first target. Chorioallantoic transplants of heart are being compared with those of spleen and liver, and the time course of their effects on the weight and the histology of the host organs is being determined.

A Humoral Factor Regulating Organ Regeneration

Charles D. Steuart, Josiah Macy Jr. Foundation Scholar in Reproduction, continued, under Dr. Ebert's guidance, to examine the proposition that organ growth is regulated by the presence in the blood of a tissue-specific inhibitory substance. According to Saetren, the compensatory growth of the rat kidney after partial nephrectomy is inhibited by a thermolabile, nondialyzable substance extractable from the kidney itself. Steuart has confirmed Saetren's finding that a peak of mitotic activity occurs in the remaining kidney

stump within 48 hours after subtotal nephrectomy. The data, summarized in table 2, show that after removal of one and one-half kidneys the remaining tissue undergoes extensive mitosis.

Next, experiments were designed to determine the type of kidney preparation that would have the greatest effect on the mitotic rate but the least toxicity. Macerates prepared in the Waring Blender are extremely toxic, killing all the recipients within 24 hours. Macerates prepared with scissors were less toxic but had little influence on the 48-hour mitotic peak. A kidney homogenate prepared with 2 vol-

TABLE 2. Mitosis in Remaining Kidney after Subtotal Nephrectomy

Interval after Nephrectomy, hours	No. of Animals	No. of Mitoses per 12,000 Cells
0	5	0
24	2	6
36	3	51
48	6	90
60	4	37
72	3	28
84	2	18
96	4	26

umes of 1.15 per cent KCl in a glass mortar with a teflon pestle had a greater inhibitory effect but also a high level of toxicity. Homogenates prepared with a teflon pestle in 2 volumes of 0.30 *M* sucrose have a high level of inhibitory activity and an extremely low toxicity, as shown in table 3. The effect of the time of injection has been studied also. Preliminary results indicate that the greatest inhibition is obtained by introducing the homogenates between 30 and 18 hours before the 48-hour mitotic peak. There seems to be no significant difference between intraperitoneal and subcutaneous injections of homogenate. Steuart then tried to determine whether the amount of kidney homogenate injected would influence the degree of inhibitory effect obtained. The results, though remarkably inconsistent, indicate that smaller

amounts do not have as large an effect on the mitotic peak as amounts equal to or greater than the amount of kidney removed from the animal.

Is the effect of kidney homogenate specific? Steuart has begun to test the organ specificity of the inhibitory effect of kidney homogenates. One and one-half kidneys were removed from a number of rats in

TABLE 3. Inhibition by Kidney Homogenates of Mitotic Activity in Kidney of Partially Nephrectomized Rats

Type of Homogenate	No. of Animals	Per Cent Survival	Per Cent Inhibition at 48-Hour Mitotic Peak
Waring Blender preparation	8	0	...
Scissors macerate	6	80	55
Saline homogenate	12	50	67
Sucrose homogenate	7	100	75

TABLE 4. Specificity of Effect of Organ Homogenates in Suppressing Mitosis

Type of Homogenate	No. of Animals	Time of Injection, hours after nephrectomy	No. of Mitoses per 13,000 Cells
No injection	6	...	116
Sucrose only	6	30	106
Liver homogenate	6	30	64
Kidney homogenate	7	18	23

four groups: one group had no treatment, and the other three groups were given injections of sucrose, liver homogenate, and kidney homogenate, respectively. The injections were made at 18 and at 30 hours postoperatively. Mitotic counts were made by microscopic examination of histological sections which had been coded and masked. The first results indicate that kidney homogenate is more specific than either sucrose or liver homogenate in suppressing the mitotic activity in the re-

maining kidney stump of the nephrectomized rat. Data are given in table 4.

Finally, to test the hypothesis that the wave of mitotic activity is a result of the removal of the kidney from the auto-regulation of its own inhibitory products, Steuart has begun experiments on animals that have been united surgically in parabi-

osis. Paired and triplet animals are cross-circulated by capillary anastomosis. When both "end" animals of a triplet are totally nephrectomized, the kidneys in the intact middle animal undergo a burst of mitosis. These experiments suggest that, in this situation, the mitotic peak is greater at 72 hours postoperatively than at 48 hours.

TABLE 5. Effect of Nephrectomy on Mitosis in Kidneys of One Member of Parabiotic Pairs and Triplets

Combination	Cases	No. of Kidneys Removed	Interval after Nephrectomy, hours	No. of Mitoses per 13,000 Cells
Triplets	1	4	48	0
Triplets	1	4	72	10
Pairs	3	3	48	10
Pairs	4	3	72	24
Single animals	7	0	0	0.3

EFFECT OF HORMONES IN DEVELOPMENTAL SYSTEMS

Sex Differentiation

Dr. Robert K. Burns' program of experimental studies on the differentiation of the gonads in opossum embryos was continued in Florida and in the home laboratory. In the course of this work it has been necessary, over the past several years, to undertake the trapping of adult opossums in considerable numbers during the late winter breeding season. Many adults of both sexes have been captured and marked, and many of them have been recaptured repeatedly during the same season. It is very rare, however, to recapture a marked animal the following year, indicating that there is almost a complete turnover in the breeding population within the span of a year. Although the majority of captured females taken in the wild have young which are too old for experimental use, long-continued trapping in the same area has resulted in the accumulation of interesting data on the reproductive history of the opossum in northern Florida. Some of this information was published in brief form two years ago (*Bulletin de la société zoologique de*

France), and additional data appeared recently in *Revue suisse de zoologie* in a paper entitled "Observations on the breeding of the American opossum in Florida."

In view of the scarcity of published data on the breeding of the opossum under natural conditions, a few points of general interest may be noted briefly as follows: (1) The first breeding season (as distinguished from a second one, which may occur in late spring or early summer) is strongly concentrated in time, more than 80 per cent of all litters being born during the first 2 weeks of February; (2) females released after being deprived of their first litters almost without exception breed again promptly, at an average interval of about 6 days after losing their first young; (3) the average number of young per litter in Florida is smaller than in other regions of the United States for which data are available, being only 6.3 per litter as compared with $9 \pm$ for the latitude of Missouri and Iowa; (4) there is little or no correlation between the weight and relative maturity of the mother and the number of young she carries in the pouch; young

females of the preceding year, when less than half adult size, carry litters as large, on the average, as those of fully grown females.

During the year experiments were continued on the effects of administration of the female sex hormone estradiol dipropionate on the histological differentiation of the gonads of embryos born at *stage 34*, McCrady's series. Since previous results had revealed that relatively small doses of the hormone must be employed if germ cells are to survive in the cortical zone of transformed testes, the dosage was again reduced to levels from 0.2 to 0.1 gamma per day. With such doses much better cortical differentiation was obtained than in earlier experiments. In thickness of the cortex as a whole, and in number of oocytes and ovogonia, the transformed testes closely approach the structure of normal ovaries of the same age, from which they can always be distinguished, however, by remnants of testis structures that survive in the medullary area. These remains show considerable variability even in individuals of the same experimental litter. In some animals the testis component is so well preserved that the gonad must be classified as an ovotestis; in others only traces of testicular structure remain and the transformed testis becomes virtually an ovary.

As in earlier experiments, the female hormone has no transforming effect on ovaries, which are essentially unchanged as far as their histological character is concerned. There is, however, some retardation of growth (explainable on the basis of a depression of gonadotrophic activity by the administered hormone) and usually a reduction in the number of germ cells in the retarded ovaries, comparable to that seen in the cortex of transformed testes. It would appear that in both organs the number of germ cells is influenced, directly or indirectly, by the dosage of the hormone.

Experiments have been renewed in an effort to induce a comparable reversal of

differentiation in the embryonic ovary by means of the male hormone testosterone propionate. Experiments in which this hormone was administered in dosages comparable to efficacious amounts of the female hormone have hitherto proved unsuccessful, for none of the parts of the embryonic genital tract showed any reaction to the hormone—an indication that the dose was probably too low. New experiments were tried during the past season in order to explore a wider range of dosages. The results are not yet known, as the material has not been studied histologically. The problem will certainly require further study. Possibly the opossum ovary may prove to be refractory or highly resistant to transformation by hormone action. It has been established that in bird embryos the ovary is much more resistant than the testis to experimental transformation by hormone action, whereas in amphibians as a rule the reverse is true. Perhaps it is only a question of determining empirically the proper experimental conditions for reversal of the ovary in this species.

Response of the Mesenchymal Tissues of the Guinea Pig to Estrogens

With the help of Marianne Jacobs Moore, Dr. R. F. Ruth has continued to devote part of his time to an exploration of the effects of estrogens on the hemopoietic tissues of the guinea pig. The hemopoietic system is characterized by cellular reproduction, migration, sequestration, and differentiation to a degree that makes it practically unique among post-embryonic organ systems. It has been demonstrated that the lymphocytes of the bone marrow, which are sequestered from the blood, are derived originally from the lymph nodes, the spleen, and the thymus. According to Ottesen, lymphocytes labeled with P^{32} and reinjected consisted of two "kinds" of cells: about one-fifth of them had a mean "age" of 3 to 4 days in the circulation, and the remainder more than

100 days. These observations led to the reinjection of thymic and lymph nodal lymphocytes labeled with P^{32} . As measured by the relative specific activity of extracted DNA, the thymic lymphocytes were sequestered most actively by the spleen, and the lymph nodal lymphocytes by the bone marrow, within 24 hours after injection. This provocative experiment as well as other evidence to be discussed suggests the possibility of significant cell transfer between hemopoietic organs.

The state of the hemopoietic system is one of the more sensitive measures of the hormonal status of an animal. A decrease in the concentration of eosinophiles in the circulation is the generally accepted criterion of some hormonal activities, usually being accompanied by a decrease in the concentration of circulating lymphocytes. "Stress" mechanisms, mediated at least in large part by hormones of the adrenal cortex, also result in a decrease in mitosis and in the size of the thymus and the lymph nodes. The thymus is prominent in the young animal and becomes smaller during and after puberty. Because the lymph nodes do not show a comparable decrease in size, it is difficult to attribute the puberal and post-puberal diminution of the thymus to the hormones of "stress." Perhaps the sex hormones have a quantitatively significant effect upon the thymic lymphocytes, the most abundant cells of the thymus. Typically, these cells are packed tightly around the edges of the organ to form a thick cortex. The small size of the cells and the manner in which they are "packed" account for the high concentration of deoxyribonucleic acid (DNA) in this organ. Thymic DNA is renewed rapidly, a finding consistent with the observation that the thymic cortex typically contains many mitotic figures and with the idea that the thymus is a major source of circulating lymphocytes. Gross changes in the size of the thymus have been used as evidence of changes in the numbers of lymphocytes *in situ*, but

they tell us little about the rates of proliferation or release (or possible uptake) of lymphocytes by the organ. Dr. G. W. Bartelmez has expressed the opinion that the size of the thymus seems to vary with changes in the hormonal status of the female.

In 1889, Kurloff reported that the cytoplasm of certain blood cells of the guinea pig contain discrete granular dots which may be as large as the cell nucleus. In the same year, Foà and Carbone not only found similar bodies in cells of the blood and the spleen of the guinea pig but also observed that they are especially numerous in the spleens of pregnant females. It was nearly forty years before it was discovered that gonadectomy decreases the concentration of circulating Foà-Kurloff cells, an observation that stimulated a number of studies of the effects of hormones on the concentration of circulating Foà-Kurloff cells.

The finding by Ledingham that the injection of 1500 μ g of testosterone propionate and/or 1500 μ g of testosterone dipropionate did not increase the concentration of circulating Foà-Kurloff cells is pertinent. Subsequent injection of 9 μ g of estradiol dipropionate increased the numbers of circulating Foà-Kurloff cells, from 2 to 5 per thousand leucocytes on the fourth day after injection, to 150 to 262 per thousand on the twelfth day. Animals that did not receive injections of estrogen contained from 1 to 6 Foà-Kurloff cells per thousand on the fourth day, and from 5 to 15 per thousand on the twelfth day. Other experiments indicated that the increase in circulating Foà-Kurloff cells becomes detectable about the sixth day after injection of estradiol dipropionate and, in general, reaches a maximum before the sixteenth day. Ledingham confirmed earlier reports of the presence of only a very few cells of this type in the untreated fetus. Ten days after the subcutaneous injection of 75 μ g of estradiol dipropionate into the mother, however, he found 24 and 14 Foà-Kurloff

cells per thousand splenic leucocytes in two fetuses. These two values are comparable to Ruth's values for the untreated 6-months-old male guinea pig.

The ease with which the Foà-Kurloff cell can be induced by hormonal treatment is no more remarkable than its appearance. Its definitive characteristic is a large, spheroidal, homogeneous body capped on one side by a cup-shaped nucleus. This body appears to contain a large amount of carbohydrate, which appears not to be glycogen and which is not readily extracted by organic solvents after fixation with formaldehyde. The induction of the synthesis and secretion of complex carbohydrate excited Ruth's interest as a possible aid in the elucidation of the mechanisms of action of the estrogens. Are estrogenic effects mediated through an initial chemical reaction common to many different tissues?

There is little evidence that estrogens act directly on the Foà-Kurloff cell or its precursor. It has been reported that neither progesterone, ACTH, deoxycortisone acetate, nor adrenaline increases the concentration of circulating Foà-Kurloff cells. Adrenalectomy causes no change in their concentration in the circulation; hypophysectomy does not prevent the typical response to estradiol. In another approach to the demonstration of direct action, Ruth tried to stimulate the proliferation of Foà-Kurloff cells by adding diethylstilbestrol to grafts of guinea pig spleen on the chorio-allantoic membrane of the chick. Neither young nor old spleen gave any evidence of stimulation. Even when the grafts appeared well vascularized, histological sections revealed a loss of Foà-Kurloff cells and a fibrous transformation of the graft comparable to that previously reported to occur in tissue cultures. The failure to establish an effect of estrogen in an isolated system required a quantitative study of the response to estrogen in the guinea pig.

Ruth has been able to demonstrate that estrogen can induce a great increase in the

concentration of Foà-Kurloff cells in the spleen. Although the spleen does not appear to be the origin of the Foà-Kurloff cell, it is suitable for chemical analyses.

The Foà-Kurloff cell provides just one expression of the great sensitivity of the guinea pig to estrogen. The guinea pig is one of the few animals in which prolonged treatment with estrogen regularly induces large and numerous tumors in nonreproductive tissues. Such tumors regress after treatment with estrogen is stopped. In some animals, at least, the proliferation of the uterine epithelium that follows treatment with estrogen is accompanied by a profuse infiltration of the endometrium by leucocytes. For example, an increase in the number of macrophages in the uterine horns of the guinea pig during the follicular phase of the estrus cycle has been described. Total removal of the guinea pig uterus prolongs the increase in circulating Foà-Kurloff cells induced by a subsequent injection of estradiol. These observations suggest that the Foà-Kurloff cell, and possibly other leucocytes, may be sequestered by the proliferating endometrium.

The statement that estrogen stimulates the phagocytic activity of the reticuloendothelial system is based largely on the increase in the number of stained bodies present in the spleen of the guinea pig after multiple administrations of estrogen and trypan blue. Photographs of these bodies cannot be distinguished from photographs of large, definitive Foà-Kurloff bodies. It has been known for over 50 years that Foà-Kurloff bodies stain with vital dyes. Since phagocytes are defined, in part, by their ability to accumulate vital dyes, it might be tempting to think of the Foà-Kurloff cell as a large phagocyte or macrophage. It is impossible to consider the Foà-Kurloff body a simple accumulation of a dye, however, for it can easily be demonstrated in the absence of a vital dye. The proliferation of Foà-Kurloff cells that can be induced by estrogen occurs in the absence of vital dyes. Moreover,

the macrophages of the spleen of the guinea pig have a characteristic morphology quite unlike that of the Foà-Kurloff cell. These differences can be clearly demonstrated in one microscopic field.

The macrophages of the spleen of the guinea pig, like those of other animals, frequently contain carbohydrate, which appears as heterogeneous and weakly stained masses of irregular shape and diffuse boundaries, in contrast to the Foà-Kurloff bodies, which stain intensely, are round or ovoid, and have smooth, regular surfaces. Some of the bodies contain an unstained area or vacuole, but otherwise they are, with rare exceptions, homogeneous. The cell nucleus is compressed by the large Foà-Kurloff body into a cup-like shape.

In table 6 are recorded the number of Foà-Kurloff cells per thousand nuclei in the spleens of young female guinea pigs in which were implanted subcutaneously either (A) a pellet of cholesterol or (B) a pellet of diethylstilbestrol. Each animal of group A was paired with an animal of group B for treatment and sacrifice. The pairs of animals were sacrificed at 1-day intervals from 1 to 10 days after implantation. The pellets, which weighed approximately 2 to 3 mg each, were not recovered. The counts of Foà-Kurloff cells were made in sections stained with the Himes and Moriber triple stain for DNA, carbohydrate, and protein. After the original labels of the microscopic slides were covered, the slides were randomized, coded, counted, and finally decoded after completion of the counts.

A substantial increase in the concentration of splenic Foà-Kurloff cells is induced by a pellet of diethylstilbestrol. The lag time of about 6 days is comparable to that obtained by Ledingham for the blood. In contrast, it will be seen that the treatment that produces a substantial increase in the concentration of splenic Foà-Kurloff cells has little effect on the concentration of splenic cells containing amorphous carbohydrate.

The data in table 7 indicate that small amounts of estradiol, comparable to those reported to elicit strong responses in the blood, do not appreciably increase the concentration of Foà-Kurloff cells in the spleen of the young male guinea pig. In this experiment, again with paired animals, the guinea pigs in group C received a subcutaneous injection of sesame oil; those in group D received a subcutaneous injection of sesame oil containing 10 μ g of

TABLE 6. Foà-Kurloff Cells and Cells Containing Amorphous Carbohydrate per Thousand Splenic Nuclei in Pairs of Female Guinea Pigs Treated with (A) Cholesterol or (B) Diethylstilbestrol

Interval after Im- plantation, days	Foà-Kurloff		Amorphous Carbohydrate- Containing	
	Group A	Group B	Group A	Group B
1	29.1	39.4	10.3	7.8
2	18.6	24.2	17.6	17.4
3	14.1	52.5	10.3	19.1
4	29.1	60.0	19.5	12.2
5	43.9	4.9	10.0	9.9
6	20.4	14.6	9.7	10.1
7	48.6	180.0	6.9	15.6
8	31.6	122.9	5.7	8.3
9	37.3	73.5	22.6	1.0
10	51.9	104.4	6.7	25.9
Total	324.6	676.4	119.3	127.3
Mean	32.5	67.6	11.9	12.7

estradiol per kilogram body weight. Note also that small amounts of estradiol do not alter appreciably the number of splenic cells that contain amorphous carbohydrate. The concentration of Foà-Kurloff cells in the spleen of the young female exceeds that of the young male; in contrast, the concentration of cells containing amorphous carbohydrate in the spleen of the young male exceeds that of the female.

To answer the question whether the concentration of splenic Foà-Kurloff cells varied with the method of administration of the hormone, the effect of an estrogen implanted directly into the spleen was

examined. The data in table 8 indicate that the concentration of neither splenic Foà-Kurloff cells nor cells containing amorphous carbohydrate is affected by the proximity of the source of estrogen.

Young female guinea pigs again were paired; group G received a pellet of cholesterol implanted in the spleen and a pellet of diethylstilbestrol implanted under the skin, and group H received a pellet of

TABLE 7. Foà-Kurloff Cells and Cells Containing Amorphous Carbohydrate per Thousand Splenic Nuclei in Pairs of Male Guinea Pigs Treated with (C) Sesame Oil or (D) Estradiol in Sesame Oil

Interval after Implantation, days	Foà-Kurloff		Amorphous Carbohydrate-Containing	
	Group C	Group D	Group C	Group D
1	1.0	2.0	57.2	32.5
2	1.9	2.0	57.0	42.0
3	8.4	4.8	71.9	42.1
4	3.8	9.0	15.4	41.8
5	8.3	12.1	32.2	49.5
6	18.0	10.5	60.0	22.7
7	20.9	5.8	30.9	55.0
8	7.8	15.2	40.7	21.8
9	3.8	13.6	18.7	40.7
10	2.8	2.0	13.1	11.0
11	0.0	21.5	1.9	32.2
12	0.0	4.9	0.8	3.0
13	21.3	10.3	30.6	26.3
Total	98.0	113.7	430.4	420.6
Mean	7.5	8.7	33.1	32.4

diethylstilbestrol implanted in the spleen and a pellet of cholesterol implanted under the skin. The method of implanting the pellet into the spleen with a blunt cannula was suggested by Arthur G. Rever.

The results reveal a considerable variation between pairs and a remarkable duplication within pairs. The greatest difference between the two members of any pair is found in one pair of animals recovered on the forty-seventh day, a result due at least in part to the inclusion of a large portion of scar tissue in some of the

histological sections. The variation between pairs suggests that the concentration of Foà-Kurloff cells in the spleen at any one time, after prolonged exposure to estrogen, cannot be predicted readily. The remarkable duplication of the values for splenic Foà-Kurloff cells within pairs is not accompanied by a comparable duplication of the values for cells containing amorphous carbohydrate.

In view of the well known sensitivity of the reticuloendothelial system to stress, it

TABLE 8. Foà-Kurloff Cells and Cells Containing Amorphous Carbohydrate per Thousand Splenic Nuclei in Pairs of Female Guinea Pigs Treated with Diethylstilbestrol (G) Subcutaneously or (H) Intrasplenically

Interval after Implantation, days	Foà-Kurloff		Amorphous Carbohydrate-Containing	
	Group G	Group H	Group G	Group H
10	88.8	72.5	10.7	13.2
34	271.1	254.8	64.5	27.0
35	348.3	244.6	71.6	32.2
35	191.7	187.2	28.7	20.1
42	210.9	211.6	50.8	37.9
46	54.6	60.1	16.9	29.4
47	27.4	138.5	4.7	45.9
47	244.2	258.2	51.0	75.4
Total	1437.0	1427.5	293.9	281.1
Mean	179.6	178.4	36.7	35.1

appeared important to determine whether the increase in concentration of Foà-Kurloff cells might be influenced by adverse conditions of diet or stress. In table 9 are presented the number of Foà-Kurloff cells per thousand nuclei in the spleen of two groups of young male guinea pigs. Group E received the usual diet of commercial pellets, but ground with 0.2 per cent 2,4-dinitrophenol, and minus the usual added greens. Group F received the same altered diet plus a subcutaneous pellet of diethylstilbestrol. Both groups received two supplements of 100 mg of vitamin C per animal and a small amount of whole pel-

TABLE 9. Foà-Kurloff Cells and Cells Containing Amorphous Carbohydrate per Thousand Splenic Nuclei in Male Guinea Pigs Fed a Diet Including 2,4-Dinitrophenol and Given (E) No Further Treatment or (F) Diethylstilbestrol

Interval after Implantation, days	Foà-Kurloff		Amorphous Carbohydrate-Containing	
	Group E	Group F	Group E	Group F
2	1.0	0.0	23.3	34.6
2	7.5	8.1	62.9	54.8
5	0.9	21.3	6.6	35.0
7	0.0	86.2	34.7	27.4
9	7.8	106.4	19.6	14.1
11	1.9	167.2	14.0	4.8
13	9.3	160.2	26.0	102.5
Total	28.4	549.4	187.1	273.2
Mean	4.6	78.5	26.7	39.0

increase in the concentration of splenic Foà-Kurloff cells within 7 days, even under adverse conditions, has little effect on the concentration of splenic cells containing amorphous carbohydrate.

Finally, it is pertinent to examine the number of Foà-Kurloff cells in the thymus after a comparable treatment. The data in table 10 were obtained from the same animals considered in table 9, i.e., those on a diet including 2,4-dinitrophenol. The animals in group E did not receive estrogen; the animals of group F received a subcutaneous pellet of diethylstilbestrol.

A small increase in the concentration of Foà-Kurloff cells in the thymus may be induced by a pellet of diethylstilbestrol. A relation of this increase to the increase in the spleen, recorded in table 9, is hardly

TABLE 10. Foà-Kurloff Cells per Thousand Thymic Nuclei
Size designations refer to sizes of Foà-Kurloff bodies.

Interval after Treatment, days	Size of Foà-Kurloff Bodies					
	Group E			Group F		
	Small	Medium	Large	Small	Medium	Large
2	0	0	0
3	0	0	0
5	0	0	0	18.3	3.8	0
7	0	0	0	0.9	0.9	0
9	0	0	0	2.8	1.9	0
11	0	0	0	4.9	33.6	0
13	0	0	0
Total	0	0	0	26.9	40.2	0
Mean	0	0	0	4.5	6.7	0

lets scattered through the ground feed. Two pairs of animals did not survive, and the surviving animals lost considerable weight. It is clear, however, that a substantial increase in the concentration of splenic Foà-Kurloff cells may be induced by a pellet of diethylstilbestrol even under adverse conditions. Presumably this reaction is not so sensitive to factors of diet and stress as to seriously impair its utilization. The treatment that produces a substantial

proved by the numerical data at hand. The presence of so high a proportion of small Foà-Kurloff bodies in the thymus 5 days after treatment with estrogen, however, suggests a thymic origin of some Foà-Kurloff cells (if indeed the small Foà-Kurloff body is the normal precursor of the large Foà-Kurloff body).

In summary, the data indicate that estrogens evoke an increase in the Foà-Kurloff cells but no significant change in

the concentration of cells containing amorphous carbohydrate. Many of the latter cells are unmistakably macrophages, characterized by large size, large nuclei, and numerous separate heterogeneous masses of carbohydrate. The data do not exclude the possibility that the Foà-Kurloff body may be taken up by macrophages after it leaves the Foà-Kurloff cell. There is no definitive evidence that any of the constituents of the Foà-Kurloff body are synthesized by the Foà-Kurloff cell. However, the analogous appearance of similar bodies in epithelial cells is routinely considered to represent synthesis. Moreover, the progressive enlargement of the Foà-Kurloff body and the ultimate loss of its contents satisfy the morphological criteria of leucocytic secretion.

The fibrous proliferation in the vicinity of a subcutaneous pellet of diethylstilbestrol was neither as massive nor as rich in carbohydrate as that around a subcutaneous pellet of cholesterol, a finding obvious in paired animals that received the same treatment except that one member of each pair received a subcutaneous pellet of diethylstilbestrol and a splenic pellet of cholesterol, and the other member of the pair received a subcutaneous pellet of cholesterol and a splenic pellet of diethylstilbestrol. The members of these pairs had remarkably similar concentrations of splenic Foà-Kurloff cells.

Also noted was thickening of the thymic cortex after treatment with estrogen, which was quite striking on the third day after implantation of a pellet of diethylstilbestrol. It was followed, on the fifth day, by an apparent transformation of part of the thymic lymphocytes of the cortex to larger cells of the medulla, accompanied by a marked increase in the number of small Foà-Kurloff bodies.

What is the meaning of these findings? It must be admitted that their significance is difficult to assess. Most of the data are highly significant in the sense that they demonstrate not just a real, but a dramatic, effect of estrogens on this unusual cyto-

plasmic element, the Foà-Kurloff body. Inasmuch as the research is off the beaten track, it has the advantage of focusing our attention on new problems. Ruth's plan was to study the effect of estrogens on the formation of the Foà-Kurloff body under definite conditions; that goal has not been realized. The investigator is always on the fringe of the unknown, where he must be alert to alterations in course. He must have a plan, but that plan must be subject to change or termination when it becomes unrewarding. For this reason, the investigation of the Foà-Kurloff cell has been terminated—perhaps we should say set aside for the time being, for, like all problems, it is subject to re-examination after the findings are viewed by others and ourselves against the changing background of related ideas. By design, the discussion has emphasized the cytological aspect of the problem. The work has indicated, within the limits of the alternatives available at present, that the Foà-Kurloff body is evidence of lymphocytic secretion. Important questions are raised about the interactions of the lymphoid and reproductive systems, and the relations of the spleen and thymus. We are reminded that to a large extent the thymus remains an enigma. What is the role in the embryo and young animal of this organ, which regresses with the onset of maturity? Emphasis is also placed on the carbohydrates of lymphoid tissues, an area of particular interest in the Department because of the finding by Ebert and DeLanney of the accumulation of carbohydrate in the spleens of embryos that have received grafts of the homologous adult organ. We are prepared to renew the attack on the problem, to take advantage of the gains already made, but only when a more promising working hypothesis is put forward.

Chemodifferentiation of the Visual Pigments

The visible changes in complexity that cells undergo in their ontogeny reflect discrete molecular events. Considerable tech-

nical problems are posed by many examples of cellular differentiation when one tries to ask questions about their chemical bases. In this perspective the report some years ago by George Wald that the visual pigments of *Rana catesbiana* tadpoles change during metamorphosis gains significance for the embryologist, for it is a developmental phenomenon amenable to a physicochemical approach. The discrete molecular change accompanying a period of intense developmental activity offers exciting possibilities.

The visual pigments of vertebrate photoreceptors are conjugated proteins having retinene, the aldehyde of vitamin A, as their prosthetic group: porphyropsin, the visual pigment characteristic of rods of fresh-water vertebrates, has retinene-2; rhodopsin, the pigment of rods of land and marine vertebrates, has retinene-1. Retinene-2 differs from retinene-1 by the presence of an added carbon bond in the β -ionone ring. According to Wald, as the tadpole emerges from fresh water onto the land it changes its visual pigment from porphyropsin to rhodopsin.

What is the precise nature of the change? What biochemical sequence of events is responsible? What is the initiating agent, and how is it correlated with the physiology of development of the whole organism? During the year Fred H. Wilt, Predoctoral Fellow of the National Science Foundation, has continued to search for the answers to several of these questions. As was described in Year Book 56, his chief analytical tools are micro modifications of existing methods. One technical advance should be noted: visual pigments are being isolated from tadpole eyes in rather pure form by first isolating the rods by differential centrifugation of homogenized retina in 45 per cent sucrose. After the rods are washed exhaustively, they are hardened in alum and extracted with 2 per cent aqueous digitonin.

SbCl_3 in CHCl_3 reacts with the vitamins A and retinenes to give a transient blue color, the absorption maximum of which is

characteristic for each compound. Methods for assaying retinene reductase (retinal alcohol dehydrogenase) have been described. Retinene isomerase is assayed by the method of Hubbard. Chromatography of vitamin A and carotenoids on alumina-impregnated paper follows the procedure of Datta and Overell. Dr. Paul K. Brown of Harvard University kindly donated crystalline all-*trans*-retinene and neo-b retinene. Concentrated neo-b retinene solutions are also prepared in this laboratory according to the method of Brown and Wald. Other essential methodological information is given in conjunction with the results.

Histology of the larval eye. For histological study, eyes have been fixed in 10 per cent neutral formalin or Helly's fluid, embedded in celloidin, sectioned at 10 μ , and stained with either Bodian's silver technique or the triple stain for deoxyribonucleic acid, protein, and carbohydrate developed by M. H. Himes and L. Moriber, a technique now in common use in this Department.

In early post-hatching stages the eye is essentially complete in structure and organization. Development during the next 2 years consists in a general increase in tissue mass due primarily to increase in the length of rod segments, and an increase of synaptic fields, in cell number, and in cell size. The affinity of cells for silver and dyes becomes greater during this period, and the outer segments of the rods stain more strongly with periodic acid-Schiff stain. With the methods employed, no striking cytological or histological changes are disclosed during metamorphosis except the general increase in size and stainability.

The larval visual pigments. Against this relatively constant morphological background dramatic events are taking place on another level of organization. Wald's original observations on larval porphyropsin have been confirmed and extended. Attempts to extract vitamin A from eyes of newly hatched tadpoles have

been hampered by the small size of the eyes and the low concentrations of vitamin A, but satisfactory analyses have been made on the eyes of tadpoles during the first year of larval life when the eye is 1 to 2 mm in diameter. The visual pigments of these larvae display a broad absorption maximum at 515 to 520 m μ . Bleached retinas of these animals, in which retinene-2 is split off the protein during the light reaction and reduced by enzymes in the tissue, have about 0.026 gamma of vitamin A₂ per retina and 0.004 gamma of vitamin A₁ per retina. Most, if not all, of the pigment is porphyropsin.

TABLE 11. Characteristics of Larval Visual Pigments

Visual Pigment	Absorption Maximum, m μ	Standard Deviation	Range, m μ
Unbleached	513.5	2.62	509-520
Bleached	395.5	8.42	379-400
Difference spectrum	523.9	4.43	517-530

As was reported in Year Book 56, visual pigments and their vitamins A can be obtained from the eyes of second-year tadpoles. The broad flat absorption maximum has a peak at about 514 m μ (table 11). After bleaching, a new absorption maxi-

mum appears at about 400 m μ , characteristic of a predominance of retinene-2. When an aliquot of the bleached extract is transferred to chloroform, the test with antimony trichloride reveals a predominance of retinene-2. Since retinene-1 is present, however, rhodopsin must also be present in these rods. The results have been confirmed by reducing the bleached mixture with KBH₄, resulting in a new maximum at 355 m μ characteristic of vitamin A₂. An antimony trichloride reaction with this product discloses that the larval eye contains approximately 0.05 gamma of vitamin A₂ and 0.015 gamma of vitamin A₁ per retina. The larval photopigment is a mixture of about 70 to 85 per cent porphyropsin and 15 to 30 per cent rhodopsin, the rhodopsin apparently increasing slightly later in the spring. Table 12 presents data illustrating the kinds of results obtained with bleached retinas and pigmented layers of the eye after extraction with diethyl ether or benzene. There is always some vitamin A₁ present in the pigmented layers, and, as Wald suggested, it is usually (although not invariably) present in a greater percentage than in the retinas.

Artificial stimulation of photopigment conversion. Wald reported that tadpoles undergoing natural metamorphosis showed changes in the composition of the mixture

TABLE 12. Vitamin A Distribution in Bleached Retinas and Pigmented Layers

Date	Age of Tadpole, years	Hind Limb/Tail	No. of Eyes	Retinas			Pigmented Layers		
				A ₂ , gammas	A ₁ , gammas	%A ₁	A ₂ , gammas	A ₁ , gammas	%A ₁
4/25/58	1	...	36	1.14	0.172	13.1	0.568	0.182	24.6
			36	1.06	0.264	20.0	0.508	0.072	12.2
12/10/57	2	...	50	0.530	0.114	18.0
4/2/58	2	0.152	19	1.20	0.281	19.0	0.478	0.190	28.4
			19	0.588 *	0.139 *	19.1	0.459	0.111	19.2
4/3/58	2	0.031	20	0.815	0.254	23.7	0.396	0.139	26.0
			20	0.762	0.276	26.6	0.490	0.172	25.9
4/16/58	2	0.040	25	0.409	0.107	20.6
			25	0.625	0.240	29.2
4/26/58	2	0.024	17	0.612	0.240	28.2	0.272	0.054	16.5

* Aliquot only.

of visual pigments: rhodopsin comes to predominate as the tadpole finishes metamorphosis. Does thyroxin, the hormone known to initiate and stimulate amphibian metamorphosis, play a role in visual pigment changes? Metamorphic changes can be induced by injections of 5 to 15 lambdas of 0.5 per cent agar saturated with L-thyroxin or by placing larvae in a dilute solution of thyroxin. By these procedures it was shown that the visual pigments were transformed during thyroxin stimulation. In table 13 are given representative data, from a large number of determinations on stimulated animals, that demonstrate clearly the changes in the visual pigments.

Therefore, experiments have been carried out to determine the site of action of the hormone in photopigment conversion. Two principal possibilities have to be considered: thyroxin might act by changing the carotenoid metabolism of the liver, a change that would be reflected in the nature of the visual pigments; or it might act on the eye itself, either on the pigmented cells, or the rods, or both.

Tests of the ability of liver and ocular tissues to metabolize possible vitamin A₂ precursors have already been reported, no activity having been detected under a variety of conditions. Analyses of the carotenoids of gut and liver of premetamorphic

TABLE 13. Thyroxin Stimulation of Photopigment Conversion

Date	No. of Eyes	Thyroxin Administered	Duration of Stimulus, days	Hind Limb/Tail	Absorption Maximum, mμ	Retinene-1, %
1/28	16	In water, 1/3-5 million	13	0.202	505	35.9
2/21	20	In water, 1/10-25 million	14	0.63	505	52.5
2/22	24	In water, 1/10-50 million	14	0.236	510	46.1
3/12	34	Injected	5	0.179	510	34.0

As will be discussed in detail later, Wilt has found it possible by placing thyroxin in the eye itself to stimulate photopigment conversion before other marked metamorphic changes occur. Thyroxin appears to be more effective in the spring than in the winter months, but this suggestion awaits more careful study. Thyroxin stimulation takes place even if the animals have been starved for several weeks and receive no food during the treatment; hence photopigment conversion can hardly be due to a change in the diet as Morton has suggested.

The tissue-specific action of thyroxin. Thyroxin stimulation of the conversion implicates the hormone directly in the process. Other phases of metamorphosis that have been studied have revealed a local tissue-specific action of thyroxin.

tadpoles also were described in Year Book 56. If thyroxin modified the pattern of carotenoid and vitamin A distribution in the liver, it might serve to indicate the importance of the liver in this system. Consequently, total carotenoids and vitamin A have been extracted from livers of animals in various stages of thyroxin-induced metamorphosis. Aliquots were saponified, and both saponified and unsaponified extracts were subjected to a battery of tests: partition in methanol-water-petroleum ether solvent systems to determine epiphasic and hypophasic carotenoids, fractionation on alumina columns, spectroscopic examination of various fractions from 220 to 550 mμ, reaction of fractions with SbCl₃ to determine vitamin A, and chromatography on alumina-impregnated paper. No vitamin A₂ has ever been

detected in these liver extracts. Vitamin A₁ is esterified and remains fairly constant in amount during the stages examined. Epiphasic carotenoids and hypophasic carotenoids are about equal in amount and remain so during stimulation by thyroxin, and the pattern of chromatographic fractions remains identical throughout stimulation. This constancy of liver carotenoids and vitamin A, when considered together with the lack of vitamin A₂, provides evidence that the liver is not primarily involved in this system, but rather that thyroxin acts elsewhere, presumably on the eye.

One way to establish the direct action of thyroxin on the eye is the use of local implants of thyroxin, a method employed in 17 experiments on over 400 tadpoles. From 5 to 15 lambdas of warm 0.5 per cent agar saturated with thyroxin is injected into the vitreous chamber through an incision made in the sclera. In the opposite eye the operation is performed but no injection is made. After a suitable period of time, injected and control eyes are recovered and the visual pigments, the retinenes, and vitamins A are compared. Although the experiments have not proved to be decisive in answering the specific question under investigation, results of interest were obtained.

The local application of thyroxin is highly effective in stimulating photopigment conversion. After 5 days of stimulation, second-year larvae show a tremendous stimulation of conversion even though metamorphosis is in very early stages as judged by other criteria. Thyroxin stimulation is also very effective in tadpoles that are only in their first year of larval life. With stimulation periods of 5 to 14 days, however, no difference between absorption maxima of photopigments from operated and control eyes was noted even though conversion was occurring in both sets of eyes. Evidently the thyroxin quickly gains access to the circulation. No reproducible stimulation of retinene-1 formation in the injected eye was found. Some ex-

periments seemed to show a stimulation of vitamin A₁ accumulation in pigmented layers of the operated eye, but the results were not reproducible. The extreme effectiveness of the treatment, the stimulation of very young eyes, and possible stimulation of pigmented-layer vitamin A₁ accumulation all suggest but do not prove a direct local action of thyroxin. Wilt is now attempting to culture the whole eye or retina of the tadpole *in vitro*.

Definition of the molecular basis of photopigment conversion. Some of the preliminary attempts to elucidate the mechanism of the photopigment change which were reported last year have been extended. It was pointed out that three macromolecular components of the visual cycle might change their catalytic specificity in such a way as to result in conversion. The enzyme retinene reductase, which reversibly converts retinene to vitamin A, is essential for supplying retinene, hence, new photopigment. A change in the specificity of this enzyme from vitamin A₂ to vitamin A₁ during metamorphosis might be responsible for the conversion. This idea has been tested repeatedly by assaying for the ability of the larval enzyme to convert retinene-1 to vitamin A₁ in the presence of DPNH. This enzyme is quite active toward retinene-1; the hypothesis is not confirmed. Wald also found that reductases from adult frog and perch retinas acted equally well on vitamin A₁ and A₂.

The same type of argument can be extended to a second enzyme that isomerizes the retinene to the correct *cis* isomer (neo-b), which can then couple to the protein moiety to form new visual pigment. This reversible reaction, neo-b retinene-1 to *trans*-retinene-1, can be examined in both directions, under different conditions that change the position of the pseudo-equilibrium. Incubation in the dark of neo-b retinene-1 with phosphate buffer extracts of premetamorphic retinas results in almost quantitative conversion of the *cis* to the *trans* isomer. Likewise, incubation of *trans*-retinene-1 with larval enzyme in

the presence of nonisomerizing yellow light results in detectable formation of neo-b retinene-1 when assayed by the iodine isomerization method of Wald and Burg. These experiments make clear that larval retinene isomerase can isomerize retinene-1 as well as retinene-2.

The hypothesis of changing specificity can be tested on the visual protein itself. The specificity of the opsin for retinene-2 might change to specificity for retinene-1 during metamorphosis. Wald had reported previously that adult frog opsin, which normally has retinene-1 as its conjugate, can couple with retinene-2 to form a new pigment with a maximum at 512 m μ , which is to the red side of the normal maximum. Conversely, Wilt reported last year that bleached visual pigment of pre-metamorphic tadpoles will form a photolabile pigment if incubated in the dark with a large excess of a mixture of retinene isomers. The use of Dartnall's method of partial bleaching and the availability of retinene preparations with a high concentration of the neo-b form have permitted a more careful investigation of this point.

In a typical experiment, an extract of second-year premetamorphic rods contained 22 per cent rhodopsin and 78 per cent porphyropsin. An aliquot of this material was bleached in two successive stages, first with light of wavelength greater than 610 m μ and then with light of wavelength greater than 550 m μ . The use of long-wavelength light revealed a true heterogeneity of photopigments and also bleached the photopigments without isomerizing the retinene, which is released from the opsin as the *trans* isomer and cannot recouple to the opsin. A concentrated solution of neo-b retinene-1 was added to this preparation and incubated in the dark for 2 hours; the difference spectrum was then recorded in the presence of hydroxylamine. Eighty-five per cent of the neo-b retinene-1 added coupled to the opsin to form rhodopsin with a difference spectrum maximum at 505 m μ . Forty-two per cent of the origi-

nal amount of opsin present formed rhodopsin.

Many experiments of this type demonstrate clearly that larval opsin does not have an eclectic specificity for retinene-2. Although there may be changes in the fine structure of opsin during metamorphosis, from the physiological point of view it remains constant. There may be, then, a true change in the metabolism of carotenoids and/or vitamin A which results in a changing availability of the prosthetic group; possibly a change in permeability barriers to the two vitamin A types may play a role.

Two other approaches to this problem may be rewarding. Radioactive compounds, especially carotenoids, may be used to disclose the precursor of vitamin A₂ and gain insight into its biosynthesis. A second long-range line of research has recently been undertaken. Immunological methods may focus attention on differences in larval and adult opsins and on detection of opsin in embryonic stages. Pilot experiments in which suspensions of beef rods are injected into adult chickens have established that a series of three injections (total nitrogen injected: 2 mg) elicits agglutinating antibodies of high titer. These sera agglutinate frog rods, and have weak activity against frog rods after absorption with frog erythrocytes and brain. These antisera and other antisera against opsin prepared in various ways may be very useful tools in examining the fine structure of visual proteins and the ontogeny of the opsin molecule.

Protein Synthesis in the Cecropia Moth

Before they attain their adult characteristics, holometabolous insects undergo a series of transformations from the egg through five larval instars and a pupal stage. Undoubtedly, many of the changes that these animals undergo are reflected in their protein constitution. Furthermore, their developmental changes are regulated by the endocrine system, which can be

controlled experimentally so that the animals develop, or remain dormant, at the will of the investigator. A number of useful experimental and surgical procedures, stemming from the work of Bodenstein, Wigglesworth, Williams, and others, are available for application in studies of insect development.

On examining a "dormant" (diapausing) silkworm pupa which is on the verge of initiating adult development (once the proper stimulus is given), one is struck by the vast reserves of stored fat. These reserves are utilized in the transformation into the adult. What are the chemical mechanisms that build up, maintain, and finally trigger the breakdown of this storehouse of energy and food reserves? (The larva stops feeding in the fall, survives, undergoes major transformations, and normally emerges as an adult the following spring.) Immunochemical procedures including the agar-diffusion methods are available to study antigenic changes in these organisms. Telfer has studied changes in certain antigens during the post-embryonic development of the *Cecropia* silkworm, being able to show that each of the antigens he considered changed its concentration independently of the others. He concluded that each has its own mode of synthesis and utilization. It might be said that progress in this area of investigation is imminent, for not only can a molecular inventory of antigenic proteins be obtained, but also an understanding of the sources and dispositions of these antigens within the developing organism, and their functions, is in sight.

Although it had occurred to several members of the Department that the developing silk moth might furnish experimental material *par excellence* for the study of factors regulating protein synthesis, the first to translate his ideas into action was Dr. Hans Laufer, who, stimulated by Dr. Howard Schneiderman, of Cornell University, has spent considerable time in exploring and adapting techniques for handling and studying the insects. The

technique of agar diffusion has proved to be of uncommon value as an exploratory tool. Dr. Laufer has been able both to confirm many of Telfer's findings and to demonstrate that certain antigenic components of the insect's blood (hemolymph) are regenerated after experimental bleeding. This observation made it even more imperative to find an independent means for identifying or distinguishing antibody precipitates, since a considerable number have been described. The question of the function of the regenerated antigens is paramount. Why do just certain molecules become replaced and not others?

Answers to a few questions have been found, but the investigation is only in its infancy. It has been shown that the antigens are proteins. It is logical to inquire next whether they have enzymatic activity. With the advice of Professor C. L. Markert, Dr. Laufer has undertaken to combine the serum agar-diffusion techniques with those of starch gel electrophoresis and histochemical identification.

He has been able to separate insect blood into a number of protein fractions, some of which have enzymatic activity. Other components have been separated which are so low in protein content as to be virtually undetectable with conventional assays. These fractions, however, were highly active as immunological antigens and enzymatically. These findings attest to the extreme sensitivity of the procedures. At least twelve different enzymatic components have been identified (about 5 drops of blood is sufficient to run all these enzyme assays). Possibly other enzymes can be resolved, separated, and identified. The enumeration of various enzymatic components in insect blood is of interest but only a preliminary to further work.

The assignment of one enzymatic activity and one antigenic activity to a particular fraction is not proof that both are properties of the same molecule, although it is highly suggestive. Of considerable significance is the direct and conclusive identification of one of these enzymes, an

esterase, as an antigen. The enzyme apparently retains its activity despite its combination with antibody in a precipitate, and can be stained by ordinary histochemical procedures in an agar-diffusion plate (fig. 7, pl. 2). Thus an enzyme can be identified histochemically while in combination with its antibodies.

Although the biological significance of Telfer's antigens "1" through "7" has always been in doubt, Laufer's findings suggest strongly that many are enzymes. Again, the identity of the antibodies used by Laufer and Telfer has not been established, but Laufer has shown that the majority of antibodies produced are identical when a number of rabbits are injected with the same antigen preparations. Using Telfer's injection procedure with a number of animals, he finds that even varying from it appreciably results in a similar antigen-antibody diffusion pattern, so that except for isolated cases we appear to be dealing with the same series of proteins (fig. 8, pl. 2).

The esterases are almost ubiquitous in their distribution, a minimum of four being present in insect blood. One indication that they may play a significant role in the development of *Cecropia* is the fact that cholinesterase cannot be found in dormant (diapausing) insects whose brains are electrically and endocrinologically inactive. When the neurosecretory cells of the brain are activated to produce the brain hormone essential for development, however, cholinesterase appears. As a matter of fact, the enzyme appears just before the onset of development.

Of considerable significance may be Laufer's finding of a change in the actual electrophoretic mobility of an enzymatic component during development. The possibility of an artifact can probably be ruled out. A stage-specific change in charge was found in a protein that maintained its biological and biochemical activity. Such a change in charge may be an alteration in the state of aggregation, ionization, etc., caused by an actual developmental modifi-

cation in an individual molecular species.

One objection that may be raised to the electrophoretic separation of enzymes is the status of several components with the same enzymatic activity. Do these represent the same protein(s) in various stages of aggregation, or are they really different? Experiments with the esterases found in insect blood demonstrate clearly that the several components have specific rates of migration and different substrate specificities, and that selective and specific inhibitors are available to demonstrate individual differences.

In pursuing the electrophoretic separation of enzymes in insects it was found that lipases had not previously been separated successfully from esterases. Because of the overlap of substrate specificities of these enzymes it was desirable to effect such separation for quantitative and kinetic studies. The separation has now been accomplished, and the possibility exists for the development of the procedure into a valuable clinical tool in the diagnosis of pancreatitis and other diseases where lipase metabolism has been disturbed.

The finding of a number of enzymatic activities in one region of the "zymogram," as Markert has called it, makes the interpretation of results difficult. Unorthodox as the idea may be, one protein may undeniably have several enzymatic activities. That is to say, the substrate specificity may be so broad or diverse as to fall into more than one classification according to the nomenclature. In Dr. Laufer's experiments, each time more than one substrate class was split, according to the electrophoretic patterns, more than one protein was found to be present by immunological procedures.

As was to be expected from earlier immunochemical studies, numerous changes in the enzymes have been recorded during the development of insects. Laufer may be able to equate the antigens with these enzymatic changes, as he has done conclusively for at least one protein. The pur-

pose of these studies is not to make a chemical inventory of the proteins and their changes in development, although

this is an obvious first step if subsequent progress is to follow, but to gain further insight into the reasons for the changes.

TERATOGENESIS

The Anatomy of Clubfoot

During the year, Dr. George W. Settle, orthopedic surgeon at the Johns Hopkins Hospital and instructor in the School of Medicine, has continued his analysis of the developmental anatomy of clubfoot. As the descriptive aspect of the study nears completion, Dr. Settle has initiated a preliminary experimental investigation of the pattern of growth in the short bones (as opposed to the long bones, which are known to grow primarily at the epiphyses). His long-range objective is to understand

how growing bones achieve and maintain their form. The fates of different regions of the growing bone are being followed by means of carbon tattooing and labeling with radioactive (tritium-labeled) thymidine.

In the course of his studies using the Collection of Human Embryos, Dr. Settle has become interested in other rare anomalies, including two congenital dislocated hips, one arthrograpodic, and two cases of metatarsus varus, which he proposes to describe.

ANATOMY AND PHYSIOLOGY OF THE UTERUS

Phases of the Menstrual Cycle and Their Interpretation in Terms of the Pregnancy Cycle

In a paper published in the *American Journal of Obstetrics and Gynecology*, Dr. George W. Bartelmez has shown that the loss of tissue during menstruation is less than is generally believed. When the interglandular stroma is stained specifically, the distinctive reticulum of the superficial zone shows that part of this zone survives throughout the duration of the flow. The thinning of the endometrium at the end of the cycle is due chiefly to loss of ground substance from the stroma, secretion from the glands, and cellular involution. The postmenstrual regeneration on which the term "proliferative phase" was based is confined practically to the epithelial cells, whereas the stroma is reorganized and the ground substance reappears during the phase of repair.

When one or more Graafian follicles begin to grow rapidly the endometrial glands produce a watery secretion which reaches its height at ovulation. It does not accumulate in the glands but is passed into the uterine lumen, presumably facilitating in-

semination. In studies on human material, endometria that are neither pro gravid nor menstruating are frequently assigned to this *follicular* phase, since the necessary data on the ovaries are usually lacking. This assignment leads to error, since some such endometria are associated with inactive ovaries and lack the characteristic secretion. In rhesus monkeys in which both ovaries and tubes are available for study, a regression can often be recognized immediately after ovulation. This finding is associated with constrictions in the coiled arteries which are the sole source of blood to the superficial zones (see volume 36 of the *Contributions to Embryology*).

Under progesterone dominance the myometrium relaxes, the rhythmic contractions change in character, and a richer secretion accumulates in the glands, producing the typical microscopic picture of the *pro gravid* phase. In pregnancy this is the period of implantation; since secretion is produced at other times in the cycle the term "secretory" for this phase is not satisfactory. Before menstruation there is always an *ischemic* phase distinguished by constriction of the coiled arteries, con-

traction of the myometrium, infiltration with leucocytes, and regression. The involution continues during menstrual ex-

travasation and after shedding of the superficial endometrium.

ANATOMY AND PHYSIOLOGY OF THE PLACENTA

Pressure Gradients Controlling Placental Circulation

In the second year of this study conducted by Dr. E. M. Ramsey, in collaboration with Doctors G. W. Corner, Jr., and W. N. Long of the Johns Hopkins Hospital Department of Obstetrics, Herbert Stran, technical assistant, and Arthur G. Rever, assistant operator, the basic prerequisite of the program, namely the monkey colony, has been strengthened. Although procurement of suitable animals grows progressively more difficult, the working colony has been enlarged and, under the expert attention of William Cleary and his associates, good health has prevailed and the unusually high conception rate of 84 per cent has been attained. Post-operative care of the experimental animals has again centered on preventing abortion and infection. Some technical difficulties in satisfactory suturing of the abdominal incision should be recorded, although the subsequent wound breakdown in 8 cases was readily amenable to treatment in all but one. No more than two observations have been possible in any animal without inducing abortion or premature delivery, a less satisfactory rate than reported previously despite intensive use of hormones in both immediate and delayed-action forms. Five animals which were used last year again conceived, and each tolerated two laparotomies very well.

The experimental techniques worked out in the first year of the study have proved increasingly satisfactory as facility in their utilization and interpretation has increased. The number of strain gauges has been doubled so that it is now possible to make simultaneous recordings of both intra-amniotic and intervillous space (IVS) pressures in two animals at once. Automatic pumps delivering 10 cc of saline per

12 hours into the uterine catheters have been introduced into the system to prevent clogging of the catheters by floating villi, small fetal parts, or coagula. Nembutal anesthesia has been used exclusively, since the surgical procedure has been more extensive than in the preliminary blood-pressure studies last year and the adequacy of reserpine and local anesthesia in laparotomy has not yet been established.

In furtherance of the initial postulate that artificial conditions should not be introduced until the normal base line was determined, no drugs have been used during the observation period. The use of hormones increasing or abolishing myometrial contractility is projected for the forthcoming experimental season not only to indicate the effect of abnormal quantities of such substances upon placental circulation but also to provide indirect evidence of their physiological role in the normal control of placental circulation. It has not been ignored that anesthesia and the operative procedures, in themselves, constitute "artificial conditions" of appreciable magnitude. Clearly, a procedure that must be introduced at an early date is the recording of myometrial contractions in the intact animal, possibly by tokodynamometer, to determine the extent of the operative disturbance.

The present report is designed only to show the scope of the observations to date. Since citation of figures so far obtained would be premature, results have been tabulated in relative terms only. In analyzing the data attention has been focused upon the following points: pressure differences determined by the stage of pregnancy; and actual values of the pressures in the amnion and the IVS and the relation between them.

Pressure differences determined by stage

of pregnancy. Reynolds described circulatory embarrassment at two epochs: first, immediately before "conversion," which is the crucial period, about halfway through pregnancy, when enlargement of the uterus by growth ceases and enlargement by stretching alone begins; second, just before onset of labor. Observations of intra-uterine pressure have, therefore, been grouped according to four periods: (a) preconversion (15 observations); (b) mid-pregnancy (35 observations); (c) end-of-pregnancy (9 observations); and (d) labor (4 as complications of b [1] and c [3]). The preconversion period can be diagnosed readily since the uterus has a characteristic spheroid shape at this time. At conversion, however, it becomes and remains cylindrical. The earlier limit of the end-of-pregnancy period must, therefore, be determined in some other way than by shape of the uterus. The investigators have elected to set this limit at 140 days since Hartman showed spontaneous delivery in the rhesus monkey to occur within the range 146 to 180 days (mean 163.7 days), but they have excluded from analysis all specimens of this group in which delivery occurred within 2 days after the operation lest incipient labor might have confused the picture. Similarly, and for the same reason, mid-pregnancy specimens are excluded from analysis if prodromal abortion contractions are suspected. No operations were performed on monkeys known to be in labor, but the condition was observed in mid-pregnancy and end-of-pregnancy animals as a late development, possibly occasioned by operative trauma.

In general it can be stated that uterine activity in the preconversion and end-of-pregnancy periods is heightened as compared with the relatively quiescent mid-pregnancy period. Thus, of 15 preconversion specimens, 10 were observed subsequently. Of these, 9 were more active in the preconversion period than at mid-pregnancy; only 1 was less active in the preconversion period. Of 9 end-of-pregnancy specimens, only 4 were without labor

for more than 48 hours. All 4 were more active at end-of-pregnancy than at mid-pregnancy. The relatively low blood levels of progesterone, shown by others to exist in preconversion and at mid-pregnancy (progesterone inhibits myometrial contractility), are doubtless pertinent. Labor is of course a period of great activity of characteristic pattern.

Actual IVS and amniotic pressure values. The relationships between amniotic and IVS pressures were obtained in 15 instances in which simultaneous recordings, suitable for statistical study, were made. In 13, the IVS pressure exceeded the amniotic pressure. Of the 2 exceptions, one was a preconversion, the other a mid-pregnancy, observation. Maximum pressures are without exception 50 mm Hg or more below systemic arterial blood-pressure levels. In the 2 cases in which amniotic pressure is greater than IVS pressure the amniotic value does not exceed 40 mm Hg, so that there is no question of inflow to the IVS being cut off. On the other hand, the reflection of myometrial contractions in the synchronous intra-uterine pressure fluctuations indicates that the myometrial contractions do obstruct venous drainage of the IVS.

In summary, the data accumulated in this second year continue to support the hypothesis advanced in previous Year Books on morphological grounds that "circulation in the maternal placenta of primates is effected by the vis a tergo of the maternal blood pressure, assisted by myometrial contractions throughout pregnancy," and to contradict the "traditional belief that the myometrial contractions 'squeeze the placenta like a sponge,' expressing its content of blood."

In addition to the new lines of inquiry mentioned incidentally in the foregoing, the investigators propose to explore the possibility of recording the pressure in the uterine arteries and veins simultaneously with intrauterine pressure and, in collaboration with Doctors Louis Hellman of

New York and Russell Morgan of the Johns Hopkins Hospital Department of Roentgenology, to inject a radiopaque sub-

stance into the uterine artery and observe its progress through the placenta by cineradiography.

APPARATUS AND TECHNIQUES

Injection of the Blood Vascular System of the Uterus

The determination of the phase of the menstrual cycle requires excellent fixation of the endometrium. Doctors I. Rossman and G. W. Bartelmez have described a procedure that makes it possible to obtain satisfactory injections of macaque uteri promptly after death.

The essential features are rapid killing with carbon monoxide and perfusion with warm aqueous NaNO_2 containing a trace of histamine. The pulse is simulated by rhythmically varying the pressure from about 54 mm Hg to 110. The most satisfactory double injections have been made by filling the entire vascular bed of the pelvis with India ink 1 part, 4 per cent gelatin

9 parts, followed by suspension of cornstarch stained by the Feulgen-Bauer method for glycogen and suspended in 2 to 5 per cent gelatin. This only rarely passes the precapillary arteries. The study of vascular patterns requires thick serial sections. The uteri are opened, fixed in formal-saline, washed, and enclosed in celloidin, or preferably in 10 per cent gelatin, frozen, and cut, mostly at 500 μ with occasional sections 50 to 100 μ .

Arteries, veins, and capillaries are completely injected except shortly after ovulation and during the ischemic and early menstrual phase. At these times, physiologic constrictions are present in many radial myometrial and/or coiled endometrial arteries.

THE COLLECTION OF HUMAN EMBRYOS

During the year, Dr. Elizabeth M. Ramsey examined 91 specimens sent by 18 doctors and laboratories from 8 states and the District of Columbia. Of the 91 specimens, 69 were discarded as of no research value, at the end of 3 months after report-

ing to the donor and in the absence of instructions to the contrary. One specimen was returned to the donor at his request, and 21 specimens had sufficient research or museum value to justify permanent preservation.

CONTRIBUTIONS TO EMBRYOLOGY

Volume 36 of the *Contributions to Embryology* which was issued on November 15, 1957, contains ten articles, the titles of which are included in the bibliographies on pages 369-371 and 457. The contents

of these articles were summarized in Year Books 54 and 55 (1954-1955 and 1955-1956). The assembling of materials for volume 37 will begin during the fall and winter, 1958-1959.

STAFF ACTIVITIES

The accomplishment of scientists is reckoned by the critical judgment of their peers. On that account it is mandatory for the members of the scientific staff to place the findings of their investigations before their colleagues. Exchange of ideas on as broad a front as possible is essential for continued vitality in pure research. Ac-

cording to the nature of their work, as well as their own interests and talents, members of the Department of Embryology accept the obligation to present their results to their colleagues and the public. In addressing themselves to the public they assume a special obligation not only to state a set of findings but also to make

clear the background of their investigations and the thinking behind them. In this connection our thoughts turn naturally to the former Director of the Department of Embryology, Dr. George W. Corner, whose writings on the history and motivations of the scientific approach have been uncommonly fruitful. The past year saw the publication of Dr. Corner's *Anatomist at Large*, an autobiography and collection of essays. Had the publication of the autobiography been delayed only a few months, the author would have been able to include another honor in addition to the many already recorded, for in the spring of 1958 he received the Passano Foundation award, in recognition of "his long and continuing researches and their many fruitful contributions to the better understanding of mammalian anatomy and physiology, with particular emphasis on reproduction."

Another embryologist long known for critical judgment and precision of thought in analytical articles and seminars was honored in March 1958, when the McCollum-Pratt Symposium was held at the Johns Hopkins University in honor of Professor B. H. Willier. Members of the Department were happy to be able to take part in this symposium, "The Chemical Basis of Development." Many members of the group took an active part in discussions; papers were given by Doctors Robert L. DeHaan and James D. Ebert.

Another symposium that attracted the attention of the staff was the Seventeenth Annual Symposium of the Society for the Study of Development and Growth, "Differentiation and Growth in Response to a Changing Chemical Environment," held at Mount Holyoke College, June 9 to 11, 1958. As President of the Society, Dr. Ebert participated in the organization of the symposium; the Department was represented by Drs. Bishop, DeHaan, Laufer, and Steinberg as well as by Mr. Beard and Mr. Coleman.

Other members of the staff also took

part in scientific meetings and described their current research in seminars and public lectures. Dr. Robert K. Burns presented lectures on the general subject of sex transformation in mammalian gonads at the Summer Institute of Radiation Biology of the University of Tennessee, the Department of Endocrinology, Medical College of Georgia, and the Conference on Experimental Embryology held at the Alligator Point Laboratory of Florida State University.

During the year Dr. Elizabeth M. Ramsey's program of conferences and lectures was unusually demanding. She took part in two conferences, "Oxygen Supply to the Human Fetus" and "Gestation," sponsored by the Josiah Macy Jr. Foundation (the former in cooperation with the Committee for International Organization of Medical Sciences), and one, "Physiology of Pregnancy in Relation to Anesthesia and Surgery," held by the New York Postgraduate Assembly of Anesthesiologists. In addition she discussed "Vascular adaptations of the uterus to pregnancy" at the Margaret Hague Maternity Hospital (Jersey City, New Jersey), and at the New York Academy of Science Conference on the Uterus, a meeting in which Dr. Bent G. Böving also took part. Dr. Böving spoke on the general subject "Implantation," and at another conference, "Endocrinology of Reproduction," held at the State University of New York, he had as his title "Endocrine influences on implantation."

Dr. David W. Bishop represented the Department at the meetings of the Biophysical Society, held at the Massachusetts Institute of Technology, and the Gordon Research Conference, at Meriden, New Hampshire. Late in June 1958, he presented a series of lectures and demonstrations dealing with the morphology and physiology of gametes and fertilization at the Summer Institute for College Teachers of Zoology, sponsored by the American Society of Zoologists.

Dr. Robert L. DeHaan lectured on early cardiac development in the Cardiovascular Graduate Research Training Program of Tulane University and the Department of Anatomy of George Washington University, and he attended the meetings of the Federation of American Societies for Experimental Biology, meetings also attended by Dr. David W. Bishop.

In addition to service as President of the Society for the Study of Development and Growth, the Director acted as chairman of the Maryland Section and a member of the Council of the Society for Experimental Biology and Medicine. During the year he continued to act as a member of the editorial board of the *Journal of Embryology and Experimental Morphology*, and was named to the board of consulting editors of *Developmental Biology*. Among the lectures given by Dr. Ebert during the year were the following: At the Marine Biological Laboratory and the Tenth Annual Scientific Meeting of the Detroit Institute for Cancer Research he discussed "The acquisition of biological specificity." At Brandeis University his subject was "Protein synthesis and macromolecular transfer in development." A general lecture, "A trail of research," was given at Miami University. The Director led conferences, "Immunology and Development" and "Chemistry of Cardiogenesis," at the Rockefeller Institute for Medical Research and Tulane University, respectively.

Several of the postdoctoral and predoctoral fellows were invited to lecture on aspects of their investigations: Dr. Hans Laufer at Cornell University, Dr. Malcolm Steinberg at Goucher College, and Mr. Fred H. Wilt at the University of Minnesota.

As in past years, the members of the scientific staff participated by invitation in the teaching programs in their areas of interest in the Johns Hopkins University and School of Medicine. Dr. Robert L. DeHaan offered a graduate level course, "Problems in developmental physiology: the cardiovascular system," in the Department of Anatomy. Dr. Mary E. Rawles took part in planning and teaching the Anatomy Department's course in embryology. Other members of the group took part in conferences in anatomy, including Drs. Bishop, Böving, Ramsey, and Ebert. Lectures were given in the following departments of the Hospital and University: Biology, Medicine, Microbiology, Obstetrics, Pathobiology, and Pediatrics. In addition, Dr. Ebert and many of the staff and graduate students took part in the embryology curriculum in the Department of Biology.

Seminars. The Embryology Seminar, organized by the Department in collaboration with Professors C. L. Markert and B. H. Willier of the Johns Hopkins University, again served as a center for the discussion of recent advances in experimental embryology. The roster of speakers included Professor H. Bautzmann, Hamburg, Germany; Dr. Elizabeth Deuchar, University College, London; Professor Z. Grodzinski, University of Krakow, Poland; Dr. Milan Hasek, Czechoslovak Academy of Science, Prague; Dr. Lionel Jaffe, Brandeis University; Dr. Cecilia Lutwak-Mann, Molteno Institute, Cambridge University; Dr. Takashi Makinodan, Oak Ridge National Laboratory; and Dr. Morten Simonsen, Fibiger Laboratory, Copenhagen.

BIBLIOGRAPHY

- Adams, W. E. Adrenal and paraganglion development in Madagascar insectivores. *Anat. Record*, 130, 261-262 (1958).
- Bartelmez, G. W. The phases of the menstrual cycle and their interpretation in terms of the pregnancy cycle. *Am. J. Obstet. Gynecol.*, 74, 931-955 (1957).
- Bartelmez, G. W. The form and the functions of the uterine blood vessels in the rhesus monkey. *Carnegie Inst. Wash. Publ.* 611, *Contribs. Embryol.*, 36, 153-182 (1957).
- Bartelmez, G. W. See also Rossman, I.
- Bishop, D. W. Reflections on recent advances in the study of the biology of spermatozoa.

- Bios*, 29, 73-81 (C. E. McClung Festschrift volume, 1958).
- Bishop, D. W. Mammalian sperm cell models reactivated by ATP. *Federation Proc.*, 17, 15 (1958).
- Bishop, D. W. See also Katsh, S.
- Böving, B. G. See Carey, M. L.
- Boyden, E. A. Bronchogenic cysts and the theory of intralobar sequestration: new embryonic data. *J. Thoracic Surg.*, 35, 604-616 (1958).
- Burns, L. M. See Burns, R. K.
- Burns, R. K., and L. M. Burns. Observations on the breeding of the American opossum in Florida. *Rev. suisse zool.*, 64, 595-605 (1957).
- Carey, M. L., L. C. Cian, H. J. Davis, B. G. Böving, and H. W. Jones, Jr. Experimental substitution of an isolated segment of ileum for the uterine tube. *Obstet. Gynecol. Survey*, 11, 156-163 (1958).
- Corner, G. W. See Heuser, C. H.
- Crowder, R. E. The development of the adrenal gland in man, with special reference to origin and ultimate location of cell types and evidence in favor of the "cell migration" theory. *Carnegie Inst. Wash. Publ. 611, Contribs. Embryol.*, 36, 193-210 (1957).
- Crowell, P. S., and C. Wyttenbach. Factors affecting terminal growth in the hydroid *Campanularia*. *Biol. Bull.*, 113, 233-244 (1957).
- DeHaan, R. L. The modification of cell migration patterns in the early chick embryo. *Proc. Natl. Acad. Sci. U. S.*, 44, 32-37 (1958).
- DeLanney, L. E. The development of the spleen of *Taricha torosa* and its experimental modification. *Proc. Indiana Acad. Sci.*, 67, 302 (1958).
- Duffey, L. M., and J. D. Ebert. Metabolic characteristics of the heart-forming areas of the early chick embryo. *J. Embryol. Exptl. Morphol.*, 5, 324-341 (1957).
- Ebert, J. D. The Department of Embryology of the Carnegie Institution of Washington. *Clin. Endocrinol.*, 6, 393-396 (1958).
- Ebert, J. D. Protein synthesis and macromolecular transfer in development. *Proc. XVth Intern. Congr. Zool., Section VII*, paper 17 (1958).
- Ebert, J. D. Review of *Methodology of the Study of Aging*, volume 3, edited by G. E. W. Wolstenholme and C. M. O'Connor. *Bull. Johns Hopkins Hosp.*, 102, 271-272 (1958).
- Ebert, J. D. See also Duffey, L. M.
- Gardner, E. See O'Rahilly, R.
- Gilbert, P. W. The origin and development of the human extrinsic ocular muscles. *Carnegie Inst. Wash. Publ. 611, Contribs. Embryol.*, 36, 59-78 (1957).
- Gray, D. J. See O'Rahilly, R.
- Greenwald, G. S. Interruption of pregnancy in the rabbit by the administration of estrogen. *J. Exptl. Zool.*, 135, 461-482 (1957).
- Greenwald, G. S. Endocrine regulation of the secretion of mucin in the tubal epithelium of the rabbit. *Anat. Record*, 130, 477-496 (1958).
- Heard, O. O. Methods used by C. H. Heuser in preparing and sectioning early embryos. *Carnegie Inst. Wash. Publ. 611, Contribs. Embryol.*, 36, 1-18 (1957).
- Heuser, C. H., and G. W. Corner. Developmental Horizons in human embryos. Description of age group x, 4 to 12 somites. *Carnegie Inst. Wash. Publ. 611, Contribs. Embryol.*, 36, 29-39 (1957).
- Katsh, S. *In vitro* demonstration of uterine anaphylaxis in guinea pigs sensitized with homologous testis or sperm. *Nature*, 180, 1047-1048 (1957).
- Katsh, S. Demonstration *in vitro* of anaphylactoid response of the uterus and ileum of guinea pigs injected with testis or sperm. *J. Exptl. Med.*, 107, 95-108 (1958).
- Katsh, S., and D. W. Bishop. The effects of homologous testicular and brain and heterologous testicular homogenates combined with adjuvant upon the testes of guinea-pigs. *J. Embryol. Exptl. Morphol.*, 6, 94-104 (1958).
- Katsh, S. See also Marshall, J. M.
- Laufer, H. Some immunochemical studies of limb regeneration in the newt. *Anat. Record*, 128, 580-581 (1957).
- Marshall, J. M., and S. Katsh. Inhibition by anticholinesterases of the electrical and mechanical activity of isolated rabbit auricles *in vitro*. *Am. J. Physiol.*, 190, 495-499 (1957).
- Marshall, J. M., and S. Katsh. Transmembrane potentials in isolated nonpregnant guinea pig uteri. *Federation Proc.*, 17, 105 (1958).
- Moffett, B. C., Jr. The prenatal development of the human temporomandibular joint. *Carnegie Inst. Wash. Publ. 611, Contribs. Embryol.*, 36, 19-28 (1957).
- O'Rahilly, R., D. J. Gray, and E. Gardner. Chondrification in the hands and feet of staged human embryos. *Carnegie Inst. Wash. Publ. 611, Contribs. Embryol.*, 36, 183-192 (1957).
- Padget, D. H. The development of the cranial venous system in man, from the viewpoint of comparative anatomy. *Carnegie Inst. Wash. Publ. 611, Contribs. Embryol.*, 36, 79-140 (1957).

- Ramsey, E. M. Vasculature of the endometrium and the placenta: a progress report. *Proc. Third Microcirculatory Conf.*, 73-77 (1957).
- Rossmann, I., and G. W. Bartelmez. The injection of the blood vascular system of the uterus. *Anat. Record*, 128, 223-231 (1957).
- Scott, R. B., and L. R. Wharton, Jr. The effect of estrone and progesterone on the growth of experimental endometriosis in rhesus monkeys. *Am. J. Obstet. Gynecol.*, 74, 852-865 (1957).
- Sensenig, E. C. The development of the occipital and cervical segments and their associated structures in human embryos. *Carnegie Inst. Wash. Publ. 611, Contribs. Embryol.*, 36, 141-152 (1957).
- Smith, E. I. The early development of the trachea and esophagus in relation to atresia of the esophagus and tracheoesophageal fistula. *Carnegie Inst. Wash. Publ. 611, Contribs. Embryol.*, 36, 41-57 (1957).
- Steinberg, M. S. On the chemical bonds between animal cells. A mechanism for type-specific association. *Am. Naturalist*, 92, 65-82 (1958).
- Wytenbach, C. See Crowell, P. S.

PERSONNEL

Year Ended June 30, 1958

(Including those whose service began or ended during the year)

Research Staff

George W. Bartelmez, Consultant in Embryology
 David W. Bishop, General Physiology
 Bent G. Böving, Physiology
 Robert K. Burns, Experimental Embryology
 Robert L. DeHaan, Experimental Embryology
 James D. Ebert, Director
 Elizabeth M. Ramsey, Research Associate and Pathologist
 Mary E. Rawles, Experimental Embryology
 Royal F. Ruth, Cytology and Immunology

Research Associates (Extramural)

Louis B. Flexner, Philadelphia
 Arthur T. Hertig, Boston
 Chester H. Heuser, Augusta, Georgia
 Samuel R. M. Reynolds, Chicago

Fellows

William E. Adams, Fellow of the Carnegie Corporation of New York
 Louis E. DeLanney, Fellow of the Carnegie Institution of Washington
 Seymour Katsh, Fellow of the Population Council
 Hans Laufer, Fellow of the National Research Council
 Jacques Mulnard, Fellow of the Rockefeller Foundation
 Malcolm S. Steinberg, Fellow of the Carnegie Institution of Washington

Visiting Investigators

Leo Clements, Omaha

Roy E. Crowder, Medical Corps, U. S. Navy
 Pieter A. DeVries, San Francisco
 Episanio E. Espinas, Louisville
 Paul Guggenheim, Omaha
 Arthur La Velle, Chicago
 Roger B. Scott, Cleveland
 E. Carl Sensenig, Birmingham, Alabama
 George W. Settle, Baltimore
 Lawrence R. Wharton, Jr., Baltimore
 Emil Witschi, Iowa City

Students (in cooperation with the Johns Hopkins University)

Robert G. Beard (graduate, biology)
 Salvatore J. Cantolino, Research Scholar, Johns Hopkins University School of Medicine (medicine)
 John R. Coleman, Fellow of the U. S. Public Health Service (graduate, biology)
 John Goffinet (medicine)
 Charles J. A. Schulte, III, Undergraduate Fellow of the National Science Foundation (undergraduate, biology)
 Charles Steuart, Scholar in Reproduction, Josiah Macy Jr. Foundation (medicine)
 Fred H. Wilt, Fellow of the National Science Foundation (graduate, biology)
 Charles R. Wytenbach, Fellow of the National Science Foundation (graduate, biology)

Clerical and Technical Staff

George Boettinger, Porter
 William E. Bouchat, Assistant Recorder
 William I. Cleary, Recorder
 Selma Cohen, Secretary

Lloyd Crane, Technician
William H. Duncan, Technician
Nancy Collier Fait, Technician
Wilbur F. Garde, Assistant Recorder
Richard D. Grill, Photographer
Wilfred D. McCleary, Technician
Marianne Jacobs Moore, Technician
Anna M. Pasterfield, Caretaker
Margaret Proctor, Secretary

Arthur G. Rever, Office Manager
Doris G. Smith, Secretary
John L. Wiser, Machinist

Special Technical Assistant pro Tempore
Joseph P. Drane

Extramural Secretarial Assistant
Edna G. Lichtenstein

DEPARTMENT OF GENETICS

Cold Spring Harbor, Long Island, New York

M. DEMEREC, *Director*

CONTENTS

	<i>page</i>
<i>Introduction</i>	375
Research	375
Staff	377
Meetings and lectures	378
Other activities	378
<i>Growth and Inheritance in Bacteriophage</i>	379
Relation between chromosomal replication and protein synthesis in phage T2	379
Genetic recombination in the presence of chloramphenicol	379
Superinfection experiments with phage lambda	381
Fractionation of T2 DNA	382
Effect of ultraviolet light on genetic recombination	383
Phage-precursor proteins	384
<i>Properties of Transducing Phages</i>	386
Host-dependent defective mutants	386
Segregation patterns of heterogenotes	389
<i>Bacterial Genetics</i>	390
Genetic recombination by transduction	390
Second-round transduction	392
Proline mutants	393
Leucine, isoleucine, valine, threonine, aspartic acid, and glycine mutants	394
Transduction of colicinogenicity	396
Mutability factor	397
Reversions among tryptophan mutants	398
Transduction technique for <i>E. coli</i>	400
Transductions in <i>E. coli</i>	400
Streptomycin resistance	403
Penicillin and chloramphenicol resistance	404
Transformation	405
<i>The Nature of the Materials of Heredity</i>	406
Patterns of cellular organization	406
Tests for mutagenic activity	410
<i>Intracellular Deoxyribonucleases</i>	412
Purification of deoxyribonuclease from salmon testes	413
"Inert" crystalline protein	414
<i>The Suppressor-Mutator System of Control of Gene Action in Maize</i>	415
The mode of operation of the <i>Spm</i> element	415
A modifier element in the a_1^{m-1} - <i>Spm</i> system	424
Continued investigation of transposition of <i>Spm</i>	428
<i>Bibliography</i>	430
<i>Personnel</i>	430

INTRODUCTION

The focal point of the research program of this Department continues to be the study of structure and properties of genes and chromosomes. These studies are being made with a variety of organisms: bacteriophages, bacteria, *Drosophila*, maize, and several other plants. Accomplishments of the past year are summarized briefly here and reported in more detail in the sections that follow.

Research

McClintock, in the course of her studies with maize, has clarified a heretofore obscure aspect of the *Spm* (Suppressor-mutator) system of control of gene action, discussed in previous Year Books. This system is known to operate at two different gene loci in maize, A_1 in chromosome 3 and A_2 in chromosome 5, both associated with the development of anthocyanin in plant and kernel. The alleles have been designated a_1^{m-1} and a_2^{m-1} . In a_1^{m-1} -carrying cultures, the independently located element of the system, *Spm*, was easily identified, and its mode of operation and inheritance behavior were readily analyzed. The operation of the other component of the system, located at a_1^{m-1} , could also be defined. Confirmable predictions could be made as to the phenotypes of plants and kernels with or without *Spm*. In the early study of plants carrying a_2^{m-1} , on the other hand, the pattern of control of anthocyanin pigmentation was perplexing. Very different phenotypic expressions often appeared in different parts of the same plant; and individuals in the progeny of such plants might also differ greatly. Because the control of gene expression in the two cases seemed so dissimilar, it was not suspected that the same system was operating in both. The confusion has now been resolved. In a_1^{m-1} cultures, the *Spm* element is active throughout the development of plant and kernel, and is stable in

its operation. In a_2^{m-1} cultures, however, the *Spm* element undergoes cyclical changes in action. The alternation of active and inactive phases during development may result in many diverse patterns of anthocyanin pigmentation, both in different parts of a plant and in different individuals of its progeny. If the stable *Spm* element from the a_1^{m-1} cultures is substituted in a_2^{m-1} cultures, the same orderly patterns of gene expression seen with a_1^{m-1} are observed. Thus, what appeared originally to be a highly complex mechanism of control of gene action has turned out to be a relatively simple one, and the apparent complexities are merely a reflection of alternating cycles of action of the controlling element *Spm*.

Kaufmann and Gay have pursued their analyses of biological ultrastructure by methods combining cytochemistry and electron microscopy. They have discovered new evidence that the chromosomes in actively dividing cells contain ribonucleic acid at all stages of the mitotic cycle. They have also explored on a theoretical basis the probable role of the blebbing phenomenon—discovered by Gay in the salivary-gland cells of *Drosophila*—in the operation of genetic systems. These explorations lead to the conclusion that many aspects of gene action may be brought into perspective when viewed in terms of the coordinating properties of the nuclear membrane. In an extension of earlier studies of the effect of near infrared radiation on crossing over in *D. melanogaster*, it has been found that treatment of young females with this agent largely eliminates the phenomenon of interference that normally exists between the white-miniature and the miniature-forked intervals in the X chromosome.

McDonald has continued her studies of the intracellular deoxyribonucleases. These enzymes appear to be correlated with synthesis of deoxyribonucleic acid and with

cell division, but their precise role in these fundamental biological processes cannot be established until they have been isolated and characterized. Her efforts this year have therefore been concentrated on ascertaining the best procedures for extraction and purification of the intracellular deoxyribonuclease of salmon testes, a tissue that was recognized last year as a very potent source of this group of enzymes. Conditions for extraction have been found which yield over 90 per cent of the total deoxyribonuclease activity, with less than 10 per cent of the water-soluble proteins, representing a tenfold enrichment by comparison with aqueous extracts of the tissue. Methods that have been developed for further purification bring about an additional sixtyfold enrichment without appreciable loss of enzymatic activity. The purified deoxyribonuclease contains at least three proteins. One of these has been crystallized, but its biological specificity has not been determined. Procedures are being worked out for further purification of the enzyme before a detailed study is undertaken of its properties, mode of action, and relation to deoxyribonucleic acid metabolism and cell division.

Hershey and his collaborators have investigated several problems concerned with the properties of phage T2, which grows on strain B of *Escherichia coli*. Their findings are summarized as follows. (1) In T2 crosses, genetic recombinants accumulate in the presence of chloramphenicol, without simultaneous protein synthesis.

(2) In a bacterium in which chromosomal replication initiated by a phage of one genotype is already under way, chromosomal replication of a superinfecting phage of a second genotype can begin without the necessity for additional protein synthesis. (3) The chromatographic properties of the DNA of T2 depend to a remarkable degree on the method of preparation of the DNA. (4) In order to account for both multiplicity reactivation of ultraviolet-irradiated phages and the effect of the irradiation on recombination frequen-

cies in phage crosses, a "group mating" hypothesis of genetic recombination and a "copy-choice" hypothesis of chromosomal replication seem to be necessary. (5) Kinetic tracer experiments show that infected bacteria contain phage-precursor protein of two kinds, one precipitable and the other not precipitable by the usual antiphage sera. Practically all the precipitable protein in the cells is phage precursor; much of the nonprecipitable protein is not. The two protein fractions seem to be formed almost simultaneously and to be incorporated into phage particles almost simultaneously. These facts suggest that phage particles are made from at least two protein subunits, each containing a large fraction of the total phage sulfur.

Demerec and his collaborators have carried forward their studies of bacterial genetics with *Salmonella typhimurium* and *Escherichia coli*. Recombination among five genetic markers in *S. typhimurium*, which can be included together in one transducing fragment, has been extensively analyzed. The results support the assumption that recombination is accomplished through a "copy-choice" mechanism, governing the exchange of characters between a recipient chromosome and a transducing fragment, and that the frequency of occurrence of "switchovers" in this process depends on the genetic constitution of the chromosome regions involved. Additional evidence of nonrandom distribution of gene loci that control related reactions is the finding that four loci concerned in threonine synthesis are closely linked, and that five loci controlling synthesis of the biochemically closely related compounds isoleucine and valine form a cluster on the chromosome. Analysis of inducibility of reversions in auxotrophs has revealed that different genera of bacteria may differ greatly in this regard; for only 14.3 per cent of 35 *E. coli* auxotrophs tested were mutagen stable, whereas 57.1 per cent of 163 *S. typhimurium* auxotrophs showed mutagen stability. Data of a study by Banič of resist-

ance to penicillin and to chloramphenicol in *S. typhimurium* show that these resistances can be transferred by transduction, and indicate that they are controlled by several gene loci. Hashimoto's findings with *E. coli* reveal that the genetic control of streptomycin resistance and dependence is vested in one gene, or two closely linked genes, and that "reversion" from dependence to nondependence is due to mutation at a suppressor locus, which is closely linked to the resistance-and-dependence locus. Fredericq has been successful in transducing the locus affecting colicinogenicity in both *S. typhimurium* and *E. coli*.

Campbell has studied the transduction by bacteriophage lambda of loci concerned with galactose utilization in *E. coli*. By segregational analysis he has obtained evidence to strengthen the conclusion that transduction in this system is actually a matter of lysogenization by a new type of genetic structure, which is part phage and part bacterial in origin. Some new phage mutants have been discovered which behave as active phages with one strain of *E. coli* K-12 and as defective phages with others. Experiments with these "host-dependent defective" mutants as markers have shown that any two transducing particles derived from separate primary events may differ in their exact content of phage loci, but that a particular particle, once formed, breeds true to type.

Staff

As a Fellow of the American Cancer Society, on leave of absence from this Department, Dr. George Streisinger spent the year from September 1957 to September 1958 working with Dr. F. H. C. Crick at the Cavendish Laboratory of the University of Cambridge, England. His place was taken by Dr. Allan M. Campbell, who, after spending the year here, is leaving to work at the Pasteur Institute in Paris as a Fellow of The National Founda-

The Department had the following fellows in 1957-1958: Dr. Kazuo Hashimoto, who held a Carnegie Institution Fellowship; Dr. Stanko Banič, Fellow of the Sklad Borisa Kidrica, Yugoslavia; and Dr. Pierre Fredericq, Advanced Fellow of the Belgian American Educational Foundation. Dr. David G. Catcheside, professor of microbiology at the University of Birmingham, and Dr. Mogens Westergaard, professor of genetics at the University of Copenhagen, were recipients of special fellowships of the Carnegie Institution, and stayed at the Department during the summer of 1958.

Our close cooperation with colleagues at the Biological Laboratory of the Long Island Biological Association has been maintained. Members of the Laboratory research staff joined us in our staff meetings and participated in our seminar lectures. The year-round research of these staff members deals mainly with genetical problems related to our studies. During the year B. Wallace and J. C. King continued their investigations of population genetics with *Drosophila*, E. Englesberg carried on work in bacterial genetics, and H. Moser continued his studies of human cells in tissue cultures.

Members of the Department again benefited by their association with visiting research workers at the Biological Laboratory. These included: Dr. E. W. Caspari, Wesleyan University; Dr. S. H. Goodgal, Johns Hopkins University; Dr. J. S. Gots, University of Pennsylvania; Dr. F. Kaude-witz, Max Planck Institute for Virus Research, Tübingen; Dr. H. Kikkawa, Osaka University, Japan; Dr. U. Leupold, University of Zurich; Dr. S. E. Luria, University of Illinois; Dr. T. Oksala, University of Helsinki; Dr. J. A. Roper, University of Glasgow; Dr. B. M. Slizynski and Dr. H. Slizynska, Institute of Animal Genetics, Edinburgh; Dr. H. Vogel, Rutgers University; Dr. D. von Wettstein, Forest Research Institute, Stockholm; and Dr. E. M. Witkin, State University of New York.

Meetings and Lectures

The twenty-third Cold Spring Harbor Symposium on Quantitative Biology met at the Biological Laboratory for nine days in June 1958. The meetings were attended by more than 250 geneticists, including 58 coming from 19 countries in Europe, Asia, and Australia. The topic of the conference was "Exchange of genetic materials—mechanisms and consequences."

Weekly meetings of the research staffs of the Department of Genetics and the Biological Laboratory were held from October to May, for informal discussion of scientific problems of general interest as well as reports of current research. Seminar lectures were also scheduled weekly throughout most of the year, and were attended by scientists from near-by institutions in addition to members of the laboratories. The speakers, who included staff members, summer visitors, and invited guests, presented reviews of completed research problems in which they had participated.

Thirty-six special seminar meetings were held during the summer of 1958, in conjunction with courses given at the Biological Laboratory. Topics related to microbial genetics and tissue culture were discussed by resident and invited speakers.

Other Activities

In connection with the *Drosophila* Educational Project, now in its nineteenth year, 1211 cultures of *Drosophila melanogaster* were sent to high schools and colleges for use in the teaching of genetics. The requests for this service came from 46 states and from Puerto Rico, Canada, Japan, and New Zealand. Mrs. G. C. Smith, Librarian, continued also as *Drosophila* stock keeper.

The November 1957 issue of *Drosophila Information Service* (DIS-31) was prepared under the direction of Demerec, and distributed early in 1958. The Department also continued to handle the preparation and mailing of *Microbial Genetics Bulletin*, which is compiled and edited by Dr. Evelyn M. Witkin, of the College of Medicine of the State University of New York. Number 15 of the *Bulletin* was issued in December 1957. The eleventh issue of *Phage Information Service*, containing abstracts of material presented at the August 1957 meeting of bacteriophage workers, was prepared at the Department under the direction of Hershey and issued in November 1957.

The report of the librarian, Mrs. G. C. Smith, records the addition of 330 volumes during the year, of which 54 were purchased, 21 were gifts or exchanges from other research institutions, and 255 were newly bound collections of periodicals. The total number of volumes catalogued in the library, exclusive of unbound publications, is now 18,798. The number of periodicals and serial publications received regularly during 1957-1958 was 413. The Department received interlibrary loan service from Brookhaven National Laboratory and Columbia University, and extended its facilities to Brookhaven National Laboratory, Long Island Agricultural and Technical Institute, Marine Biological Laboratory, Rockefeller Institute for Medical Research, Roswell Park Memorial Library (Buffalo), Rutgers University, State University of New York College of Medicine, Syracuse University, Tufts University, University of Alberta (Canada), University of Rochester, and U. S. Army Medical Research Library. A total of 343 books was borrowed by members of the staff, their assistants, and guest investigators.

GROWTH AND INHERITANCE IN BACTERIOPHAGE

A. D. Hershey, Gebhard Koch, André Kozinsky, Joseph D. Mandell, René Thomas, and Jun-ichi Tomizawa

This report deals with five topics: (1) an attempt to recognize genetic consequences of the synthesis of phage-precursor nucleic acid (DNA) in the presence of chloramphenicol, (2) an attempt to determine whether or not DNA synthesis involves transfer of information to protein or protein-containing substances as an intermediate step, (3) an attempt to separate and identify physically and biologically different fractions of the DNA of phage T2, (4) an attempt to characterize the damages produced by irradiating phage-precursor DNA with ultraviolet light, and (5) an attempt to analyze kinetically the synthesis of phage-precursor proteins in infected bacteria.

Our work is supported in part by a grant (C-2158) from the National Cancer Institute of the National Institutes of Health, U. S. Public Health Service. Thomas is a Fellow of the Rockefeller Foundation, and *chargé de recherches* at the Fonds National de la Recherche Scientifique (Belgium). Tomizawa is on leave from the National Institute of Health of Japan, and is the recipient of a Fulbright Travel Grant.

Relation between Chromosomal Replication and Protein Synthesis in Phage T2

In bacteria infected with phage T2, DNA synthesis proceeds when protein synthesis is stopped, some 5 or 10 minutes after infection, by the addition of chloramphenicol. Under these conditions DNA synthesis continues for about 60 minutes, at which time the cells contain about 130 phage-equivalent units of DNA per bacterium. If chloramphenicol is left in the culture after this time, there is a progressive leakage of cell constituents into the culture fluid, and the cells soon lose the capacity to produce phage when transferred to media lacking chloramphenicol. If the chloramphenicol is removed from

the culture 40 to 60 minutes after infection, however, protein synthesis resumes, and phage particles are formed which contain DNA synthesized before and protein synthesized after the removal of the antibiotic. Given these facts, one immediately asks whether or not the phage-precursor DNA that accumulates in the presence of chloramphenicol represents the finished chromosomes of future phage particles.

Tomizawa's experiments have demonstrated that phage-precursor DNA synthesized in the presence of chloramphenicol is subject to damage by ultraviolet light which resembles, qualitatively and quantitatively, the damage produced by irradiation of phage particles themselves (Year Book 56, p. 363). Such damage is unmistakably localized in chromosomes. Considered alone, this evidence shows quite satisfactorily that phage-precursor DNA already contains, at the time of synthesis, the genetic specifications of phage particles formed subsequently. The following experiments are also consistent with that conclusion.

Genetic Recombination in the Presence of Chloramphenicol

It has generally been assumed that genetic replication and genetic recombination are concurrent processes during phage growth. This assumption has never been proved. The following experiments tend to show that it is correct and to confirm our previous conclusion that DNA synthesized in the presence of chloramphenicol is in fact finished genetic material.

At various times, in our laboratory and elsewhere, people have tried to determine whether or not genetic recombinants accumulate in the presence of chloramphenicol when phage crosses are performed with the appropriate antibiotic treatment. Significant effects were never observed, but the interpretation of the experiments was

complicated both by the inaccuracy of the genetic methods and by theoretical uncertainty as to how big an effect should be expected. Tomizawa has now obtained significant results by employing in the crosses *rII* mutants of phage T4, kindly supplied by Chase and Doermann. With these mutants the recombination frequencies can be measured by precise selective methods because the wild-type recombinant, but not the mutant phages, can form plaques on certain strains of *Escherichia coli* (Benzer).

The cross *r59* × *r61* was performed in peptone broth in the usual manner except

recombination frequency than was observed at comparable phage yields obtained without chloramphenicol treatment. These results are reasonably consistent with the hypothesis that recombination frequency is proportional to number of generations of DNA replication in a pool of replicating and mating chromosomes, and that neither replication nor recombination is suppressed by chloramphenicol.

Frequency of genetic recombination can be greatly increased by ultraviolet irradiation of the phages before infection (Jacob and Wollman) or after infection (Burgi). This fact makes possible a somewhat dif-

TABLE 1. Effect of Chloramphenicol on Frequency of Genetic Recombination

No Chloramphenicol, Lysis at Minute t_2			Chloramphenicol Present from Minute 7 to t_1 , Lysis at t_2			
t_2	Phage per Bacterium	Recombina- tion Fre- quency, %	t_1	t_2	Phage per Bacterium	Recombina- tion Fre- quency, %
10	0.5	1.0	15	22	5	2.6
12.5	7	1.5	15	25	20	2.6
16	52	2.6	20	27	4	3.0
21	126	3.5	20	32	21	3.0
26	194	4.1	30	39	6	2.9
31	263	4.5	30	49	28	3.2
41	260	4.8	40	53	6	3.6
			40	60	19	3.9

that, to a portion of the culture, 30 µg/ml of chloramphenicol was added at 7 minutes after infection. Samples of the culture without chloramphenicol were lysed at intervals, to measure the normal recombination frequency and its dependence on time of lysis. Samples of the culture containing chloramphenicol were diluted at various times to release the inhibition by the antibiotic; and then samples of these cultures were lysed at appropriate times to permit measurement of recombination frequency. The results are shown in table 1. They reveal a small progressive rise in recombination frequency as the period of exposure to chloramphenicol was increased. Even a short period of exposure to the antibiotic yielded phages showing a higher

ferent test of the hypothesis that genetic recombination can occur in the presence of chloramphenicol. The test, suggested to us by Burgi and Streisinger, can be described as follows.

Bacteria are infected, in a glucose-ammonia medium, with two mutant phages between which genetic recombination normally occurs with low frequency. Chloramphenicol is added to the culture 9 minutes after infection. At 12 minutes after infection, the culture is irradiated with a dose of ultraviolet light (7 phage-lethal hits) sufficient to increase recombination frequency about fivefold in the absence of chloramphenicol. The irradiation does not appreciably affect DNA synthesis or phage growth under these conditions. Now, if

genetic replication occurs in the presence of chloramphenicol during the period following irradiation, it can be asked whether the products of replication will consist of chromosomes of the parental genotype only or will include recombinants as well. To determine the answer, chloramphenicol is removed from the culture 60 minutes after infection, phage particles are allowed to form, and recombination frequency among them is scored. The result shows that phage particles produced under these conditions include recombinants whose frequency depends on the dose of ultraviolet light in the same way as that of phage particles produced in cultures not subjected to chloramphenicol. It confirms in a quantitatively satisfactory way that genetic recombination occurs during the chloramphenicol period, and not exclusively after removal of chloramphenicol.

Superinfection Experiments with Phage Lambda

The most straightforward method of measuring genetic replication in phage-infected bacteria makes use of the following principle. If a bacterium is infected at time zero with a genetically marked phage (say h^+), and is then superinfected at various times with a second genetically marked phage (in this case h), the second phage makes a progressively smaller genetic contribution to the eventual phage yield as the time of superinfection is delayed. In the absence of complicating factors, the rise in the ratio of h^+ to h among the phages produced should measure the multiplication of h^+ chromosomes up to the time of superinfection. Such experiments with phage T2 were reported by Visconti and Garen (Year Book 52, p. 221). These investigators noted, however, that quantitative interpretation was difficult owing to the resistance of T2-infected bacteria to superinfection. Hershey, Burgi, and Melechen attempted to apply this method, in experiments with T2, to the demonstration of genetic replica-

tion in the presence of chloramphenicol, but failed to surmount the technical difficulties.

In the meantime, similar methods have been employed by Whitfield and Appleyard, Jacob and Wollman, and others, in experiments with phage lambda. With this phage no resistance to secondary infection develops. Thomas has therefore undertaken a study of the effects of chloramphenicol in this system. He uses virulent derivatives (c mutants) of phage lambda exclusively, making primary infections with h^+ phage and superinfections with h . His results may be summarized as follows.

1. In the absence of chloramphenicol, the genotype ratio h^+/h in the final phage yield increases rapidly as the time of superinfection is delayed. This finding is taken to mean that h^+ chromosomes begin to multiply in the cells soon after infection.

2. If chloramphenicol is added at the time of primary infection, and the cells are superinfected at various times in the presence of chloramphenicol, which is then removed to permit phage particles to form, the ratio h^+/h remains constant and equal to that found by simultaneous infection with the two phages in the absence of chloramphenicol. This observation is interpreted to mean that infection in the presence of chloramphenicol is not followed by multiplication of phage chromosomes. Such a result was to be expected since, with phage T2, DNA is not synthesized under these conditions.

3. If chloramphenicol is added some minutes after primary infection, and the cells are then superinfected, the ratio h^+/h remains constant independently of the time of removal of chloramphenicol, and is close to the ratio found by superinfection at the same time in the absence of chloramphenicol. This result is taken to mean that, if chromosomes multiply in the presence of chloramphenicol under these conditions, the primary infecting phage, which prepared the scene for multiplication, and

the superinfecting phage, which is not permitted to direct protein synthesis, participate equally in the multiplication.

4. If chloramphenicol is added some minutes after infection, and the cells are then superinfected *at various times*, chloramphenicol being removed at still later times, the ratio h^+/h increases about two-fold after the addition of chloramphenicol, and then remains constant. This result seems to show that only a limited multiplication of chromosomes occurs after the addition of chloramphenicol to cultures infected with phage lambda. Thomas does not yet have information about the effects of chloramphenicol on DNA synthesis in this system, with which the genetic results can be compared.

It should be added that the experiment described in item 3 can also be performed satisfactorily with T2, and yields an identical result, as Burgi and Melechen discovered. The results of the other experiments cited above when performed with T2 cannot be interpreted, owing to partial exclusion of the superinfecting phage.

Item 3 is the focus of interest in these experiments, because it suggests that, with either phage lambda or T2, no information transfer involving protein synthesis is necessary as a preliminary to genetic replication. With phage lambda, the result is not yet quite satisfying because chloramphenicol seems to limit rather severely the rate of genetic replication (item 4). With phage T2 there is independent evidence of genetic replication in the presence of chloramphenicol, but the superinfection method is in general technically unsatisfactory with this phage. Therefore our results tend to support the idea of autonomous replication of DNA but fall considerably short of an elegant demonstration.

It is worth recalling here the principal findings that suggested the idea of information transfer as a prerequisite to DNA synthesis. When bacteria are infected with phage and are then exposed to radiation

(ultraviolet light or decay of assimilated radiophosphorus) at various times, the phage-producing capacity of the infected bacteria proves to be sensitive to radiation damage during the first few minutes of viral growth, but soon becomes resistant (Luria and Latarjet; Stent). Chloramphenicol prevents the progress of the stabilizing reactions even under conditions in which DNA synthesis proceeds (Tomizawa and Sunakawa; Stent). In explanation of these facts, both Stent and Tomizawa suggested transfer of information to non-DNA sites of DNA synthesis by a process dependent on protein synthesis. Our results do not confirm their hypothesis, but neither do they suggest an alternative explanation of the radiobiological findings.

Fractionation of T2 DNA

A particle of phage T2 contains an amount of DNA equivalent to an aggregate molecular weight of about 100 million. Various physical measurements suggest that this represents 5 to 20 molecules of DNA. Genetic experiments show that the phage particle contains only one chromosome. How is the relation between these two numbers to be understood? Some smaller phages contain much less DNA (Sinsheimer, Tessman). Does this mean that the chromosome of T2 is a more complex structure than the chromosome of these smaller phages, or does T2 contain both chromosomal and nonchromosomal DNA? We are exploring the second alternative, which is suggested by the following facts: (1) The DNA in a particle of T2 (analyzed by autoradiography of P^{32} -labeled material) seems to consist of a single large piece and several smaller ones (Levinthal; C. Thomas). (2) The DNA of T2 can be separated into two chemically distinct fractions by adsorption to and fractional elution from a basic protein (Brown and Martin). (3) In experiments in which phage-precursor DNA is irradiated with ultraviolet light and sub-

sequently analyzed by biological methods for the presence of radiochemical damages, evidence can be obtained that the effective damages are localized in about 40 per cent of the irradiated DNA (Tomizawa). Up to now, however, it has not been possible to correlate or interpret these and other facts satisfactorily.

In order to pursue these questions further, Mandell is developing an additional method for fractionating DNA. His method is very similar to that of Brown and Martin except that he uses methylated

More recently, Mandell has investigated the dependence of observed properties of T2 DNA on methods of preparation. He has compared DNA liberated from phage particles by osmotic shock, DNA prepared by the phenol method (Gierer and Schramm; Kirby), and DNA prepared by the chloroform method. As tested by behavior on the fractionating column, the first two methods yield a very similar product, which is quite different from DNA subjected to chloroform treatment. This difference is illustrated in figure 1, which

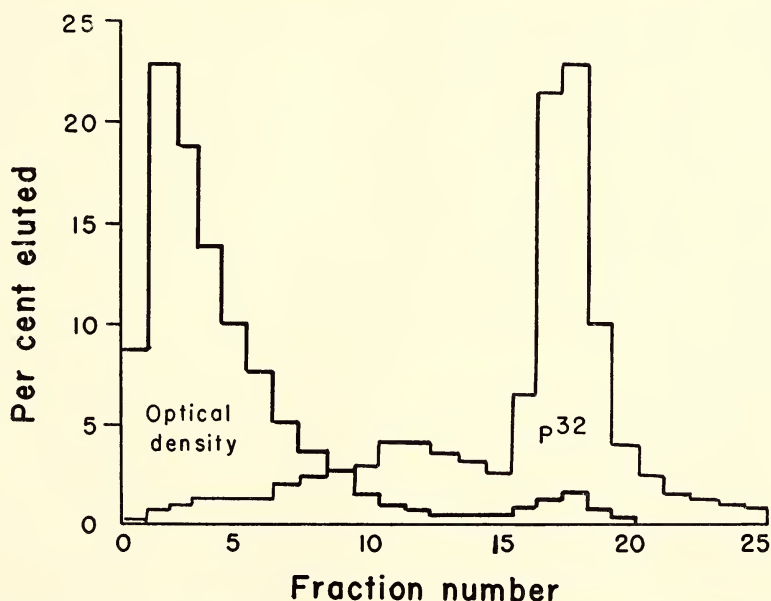


Fig. 1. Separation of P^{32} -labeled, untreated DNA from unlabeled, chloroform-treated DNA by elution with increasing salt concentrations from a column of methylated serum albumin. Concentrations of NaCl range from 0.7 M to 0.8 M.

serum albumin supported on a column of diatomaceous earth (Lerman) instead of a natural basic protein. In earlier experiments Mandell and Burgi found that valid fractions could be obtained in this way, and that the DNA of T2, for example, could be separated from that of T4; but they did not obtain any evidence of a biologically interesting fractionation of the DNA of T2. In this work they used preparations of DNA that had been deproteinized in the usual way by shaking with chloroform and octanol.

shows the elution diagram of a mixture containing a small amount of P^{32} -labeled DNA, prepared from T2 by osmotic shock, and a relatively large amount of chloroform-treated DNA. It will be seen that the two are almost completely separable. Nothing has been learned yet about the chemistry of the separation.

Effect of Ultraviolet Light on Genetic Recombination

Evidently, much of our recent work depends for its interpretation on an under-

standing of the action of ultraviolet light on DNA. Owing chiefly to the work of Doermann and his students, it is virtually certain that the effects of ultraviolet light on phage particles are due, at least in part, to localized damage to phage chromosomes. Our results further illustrate the importance of this general conclusion. Therefore we have been impelled to look in other directions for additional clues to the nature of the genetic damage.

According to one hypothesis, radiation damages produce local blocks to the replication of DNA. This hypothesis provides a ready explanation of two phenomena: "multiplicity reactivation" of irradiated phage particles in bacteria infected with two or more such particles, and the increased recombination frequency that accompanies multiplicity reactivation in crosses between irradiated phages. The hypothesis is interesting also because it seems to call for a copy-choice mechanism of recombination, as opposed to a mechanism involving breakage and reunion of finished chromosomes.

We have therefore tested the following model for the effects of ultraviolet light on recombination frequency in phage crosses. First, we assume that only radiation damage to the chromosomal region lying between or near the markers can affect recombination frequency. Second, we assume that multiplicity reactivation occurs by a copy-choice mechanism that selects undamaged chromosomal segments for replication; damage points force "switches" from one source of information to another during chromosomal replication. Third, we assume that each switch of information source involves a new random choice of partners (as opposed to switches confined to two members of a pair). These assumptions predict the following relation:

$$L = \frac{nx}{\log [(B - R_0)/(B - R)]}$$

where L is the length of the phage chromosome, x is the length of the chromosome

segment in which radiation damages force recombination between a given pair of markers, n is the number of radiation damages per chromosome, R_0 is the recombination frequency between the specified markers in the absence of irradiation, R is the dose-dependent recombination frequency after irradiation, and B (0.43 in our experiments) is the recombination frequency at genetic equilibrium. If it is assumed that the same damages effective in the inactivation of phage particles are responsible for the increased recombination frequency, all the quantities entering into the equation can be measured independently.

We have tested this theory in several crosses with T2 and T4, with considerable success, as indicated in figure 2. Needless to say, the success can scarcely be taken as strong support of all the assumptions of our theory. The results nevertheless encourage further exploration of the stated lines of thought about mechanisms of genetic recombination and effects of radiation. In particular, they suggest a "group mating" model for genetic recombination that is susceptible of independent test (Bresch; Steinberg and Stahl).

Phage-Precursor Proteins

Work in several laboratories has shown that bacteria infected with phage T2 contain, besides phage particles, several structures morphologically and serologically related to the protein coat of the particles. The status of these materials as precursors or by-products of phage growth has never been clarified. Tracer methods applicable to this question have been in the course of development in our laboratory for several years (Year Book 53, p. 210). Koch has now improved and applied them.

In his experiments he labels phage-precursor proteins with radioactive sulfur (S^{35}), and expresses his results in terms of a phage-equivalent unit of protein, which in these experiments amounts to 1.5×10^{-12} μ g of sulfur, measured as S^{35} insoluble in

trichloroacetic acid after appropriate fractionation of lysates of phage-infected bacterial cultures.

Two constituents of the lysates can be directly assayed by radiochemical methods: mature phage particles, isolated by fractional centrifugation, and "surplus antigen," precipitated from supernatant fluids by specific antiserum after removal of the phage particles. Koch finds that surplus antigen starts to form in the cells a few

and leaves it somewhat less rapidly afterward. The maximum labeling of surplus antigen is observed about 30 seconds after the end of the pulse. During the next 15 minutes, S^{35} contained in surplus antigen falls to a low minimum value concomitantly with the incorporation of S^{35} into phage particles.

A balance of S^{35} -labeled protein in all fractions of the lysates shows, however, that only about 60 per cent of the phage-

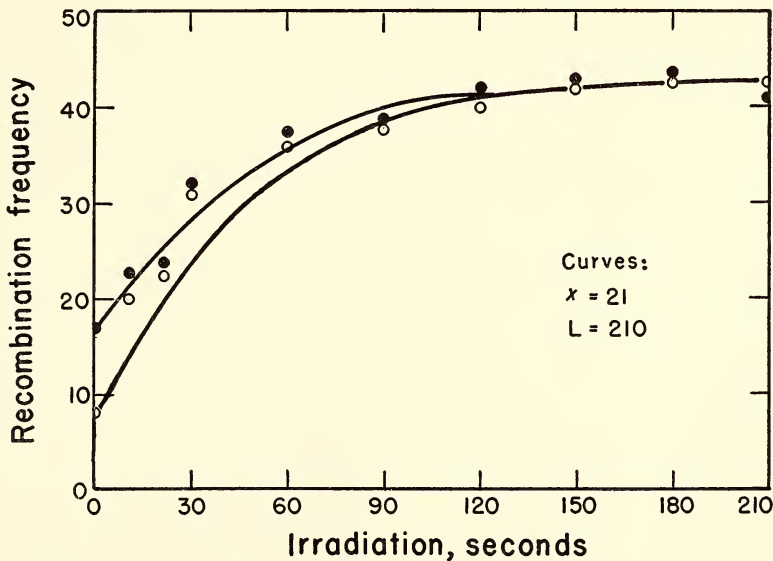


Fig. 2. Dependence of recombination frequency on dose of ultraviolet light given to infected bacteria 5 minutes after infection. Open circles: premature lysates yielding about 1 phage particle per bacterium. Filled circles: spontaneous lysates yielding about 100 particles per bacterium.

minutes before phage particles appear, and soon reaches a level of about 20 units per bacterium. This amount remains constant from the 20th to the 70th minute after infection, and then tends to increase somewhat. During the same time-interval the number of intrabacterial phage particles increases continuously, numbering about 120 per bacterium by the 60th minute.

In experiments in which a "pulse" of S^{35} is fed to the culture, for instance between the 10th and 16th minutes after infection, the phage-precursor status of the surplus antigen is readily demonstrated. It is found that S^{35} enters surplus antigen very rapidly during the feeding period,

precursor protein is precipitated by anti-phage serum. One can thus speak of "antigenic" and "nonantigenic" proteins, both of which include phage precursors in the sense that S^{35} flows out of them simultaneously with the incorporation of equal amounts of S^{35} into phage particles. In this sense, 87 per cent of the surplus antigen is phage precursor, and 50 per cent of the nonantigenic water-soluble protein in the lysates is also phage precursor.

Once S^{35} is incorporated into phage particles, all of it, of course, is precipitated by antiserum. One might expect that the incorporation of precursor proteins into precipitable structures would be a stepwise

process, more or less completed for a given phage particle in advance of the moment at which the particle achieves its finished, infective form. If this were so, the kinetic experiments should show clearly that S^{35} passes successively through a pool of non-antigenic precursor into a pool of antigenic precursor and finally into phage particles. There is in fact a suggestion of this in the

experiments described, but the bulk of the nonantigenic phage precursor seems to enter phage particles as a terminal step. This strongly suggests that phage particles contain two types of protein subunits, each comprising a major fraction of the total phage protein. It may be possible to verify this suggestion by direct analytical methods.

PROPERTIES OF TRANSDUCING PHAGES

Allan Campbell and Evelyn Balbinder

We have been studying the transduction of galactose markers in *Escherichia coli* by bacteriophage lambda (λ). In this system, as was discovered by Morse, the result of transduction is frequently the formation of unstable partial diploids (syngenotes). The nature of the transducing particles has recently been clarified by some work of Arber, Kellenberger, and Weigle, and by some of our own. These particles are not true bacteriophage but rather contain, apparently within a phage skin, an incomplete phage genome. This genome lacks an interstitial segment (*dg* region), which contains the loci *m_s* and *h*. By definition, each such particle also contains the *gal* region, derived originally from a bacterial host. What is more interesting, segregational analysis of syngenotes indicates that the phage genes and the bacterial genes which enter a bacterial cell from a single transducing particle remain associated in the prophage state. This association implies that the two are connected as parts of one genetic structure rather than being chance cohabitants of the same phage skin.

Our work this year has been directed at answering two questions. First, what is the arrangement of genetic loci within this new structure? Second, how does it originate? Neither problem is yet solved. The principal advance has been the discovery of some new phage mutants, which constitute useful tools for these investigations. We shall first describe the properties of these mutants and the information

they have so far yielded about the transductional system. Then we shall report on some studies of segregation patterns of syngenotes that strengthen our basic assumptions and seem to reveal some new phenomena for which we have no specific explanation.

Host-Dependent Defective Mutants

Bacterial strains. Wild-type *E. coli* K-12 is lysogenic for lambda. Nonlysogenic substrains are readily found among the survivors of heavy doses of ultraviolet radiation. Such "cured" strains have been made by various investigators from different K-12 stocks. Reference-type lambda phage will form plaques on all cured strains; our new mutants (host-dependent defective, *hd*) plate on one of them (C600 of Appleyard) but not on any of the others tested (112 of Wollman, W3101 of the Lederbergs, W3350 of Weigle, K-40 of Bertani). All these strains are descended from the wild-type K-12 used by Lederberg and Tatum in 1946. C600, and none of the others, was derived from a *thr leu* stock (Y-10), which was made by them at about that time. Strain W3350, which is *gal-1 gal-2*, has been employed as a standard recipient in our transduction experiments.

Crosses of C600 (F^-) or mutants thereof with 112 (F^+) or K-40 (HfrH) indicate that the pertinent difference is in a chromosomal factor closely linked with *gal* (and therefore with lambda) and transferred with high frequency by K-40. All

hd mutants plate on C600 and not on the other parent. No bacterial recombinants have been found on which some but not all mutants will form plaques.

We have relysogenized both C600 and the other strains and then cured these lysogens. Such recured derivatives always have the same properties as the parent strain from which they came. The same holds true for lambda-sensitive segregants from defective syngenotes.

Phage mutants. The mutants have been isolated as small-plaque variants of lambda on C600 obtained by the Weigle double-irradiation technique. Our standard procedure has been to pick material with a needle from the center of each small plaque appearing on the primary plates and to stab it into nutrient-agar plates, which are then incubated. The resulting mixed colonies are printed onto C600 and W3350, irradiated, and observed for the production of phage that will lyse these indicators. Those that do not lyse W3350 are further purified. The method selects for mutants that, besides being host dependent, are able to lysogenize well. Ten such mutants have been studied. Their order, determined by three-point crosses with the known markers *m₅*, *h*, and *c*, is as follows:

(*hd₁₀*, *hd₁*)-*hd₄*-*hd₉*-*hd₂*-*hd₆*-*m₅*-*h*-
hd₇-*c*-*hd₈*-*hd₃*-*hd₅*

The formation of small plaques on C600 is explainable by the fact that the mutants have a low burst size on this strain. They also have an apparent selective disadvantage in mixed infection with wild type. On W3350, both these properties are accentuated; the burst size is generally much less than unity, although with a very leaky mutant such as *hd₃* it may be of the order of 1. W3350 can be lysogenized by these mutants. The lysogens are immune and inducible; but, again, very little phage is produced after induction. The amounts, for three of the mutants, are shown in table 2.

Mixed infection of W3350 by any pair of mutants is always much more productive than infection by one alone. All combinations have been tested by mixed-spot tests, and some by one-step growth curves.

It is also possible to lysogenize W3350 with a lambdoid phage that has a different specific immunity than lambda, and then superinfect the lysogen with the mutants. We have employed for this purpose the λ -434 hybrid strain of Kaiser and Jacob,

TABLE 2. Leakiness, Reversion, and Recombination with *hd* Mutants

Bacteria and Phage	Plaque-Forming Units Produced per Irradiated Cell (or per Infected Cell)	
	Assayed on C600	Assayed on W3350
Culture induced:		
W3350 (λ <i>hd₁</i>)	4.0×10^{-3}	2.5×10^{-5}
W3350 (λ <i>hd₂</i>)	3.1×10^{-5}	5.7×10^{-6}
W3350 (λ <i>hd₃</i>)	3.1×10^{-1}	1.1×10^{-4}
Lytic infection:		
λ <i>hd₁</i> on W3350	$< 10^{-2}$	
λ <i>hd₂</i> on W3350	$< 10^{-2}$	
λ <i>hd₁</i> and λ <i>hd₂</i> on W3350	10	
λ ++ on W3350	98	
λ <i>hd₁</i> on C600	21	
λ <i>hd₂</i> on C600	13	
λ ++ on C600	99	

which we designate λ *imm*⁴³⁴. The efficiency of plating on W3350 (λ *imm*⁴³⁴) varies from 10^{-3} for λ *hd₇* to 1 for λ *hd₃* and λ *hd₅*. With all the mutants except *hd₇* and *hd₈*, the amount of growth is sufficient to allow clear-cut differentiation between W3350 and W3350 (λ *imm*⁴³⁴) in a simple cross-streak test.

The *hd₁*, *hd₂*, and *hd₃* markers have been introduced into the λ -434 stock by phage crosses. The *hd* mutants with lambda immunity can grow on W3350 (λ *hd imm*⁴³⁴), provided that the *hd* markers of the superinfecting phage and the prophage are not identical. The same is true

of the growth of λ *hd imm*⁴³⁴ on W3350(λ *hd imm* ^{λ}).

Application to the study of galactose transduction. The fact that the ability of a particular *hd* mutant to grow on a lysogenic derivative of W3350 is contingent on the presence in the prophage of the corresponding *hd*⁺ allele has given us a tool for studying the extent of the *dg* region in different syngenotes. Defective syngenotes of the type W3350(λ *gal* + *imm*⁴³⁴) are cross-streaked against the series of *hd* phages. If the λ *gal* + was derived from *hd*_{*n*}⁺ phage, we can test for the

3. *hd*₃, *hd*₅, *hd*₁₀ present; *hd*₁, *hd*₂, *hd*₄, *hd*₆, *hd*₉ absent

4. *hd*₃, *hd*₅ present; *hd*₁, *hd*₂, *hd*₄, *hd*₆, *hd*₉, *hd*₁₀ absent

If it is assumed that the *dg* region constitutes a continuous deletion of phage genetic material, the occurrence of these four types indicates the order *hd*₁₀–*hd*₁–*hd*₄–(*hd*₂, *hd*₆, *hd*₉), which is compatible with that determined by phage crosses. Evidently a λ *gal* genome, once formed, multiplies true to type. The precise amount of phage genetic material contained in such a genome is determined

TABLE 3. Transductions by Lysogenic Heterogenotes Made with Phage λ *hd*₁ *imm*⁴³⁴

Heterogenote	Transductants				Galactose-Negative Segregants			
	Number	Immunity *	Ability to Support Growth of <i>hd</i> Mutants		Number	Immunity	Ability to Support Growth of <i>hd</i> Mutants	
			Grow	Do Not Grow			Grow	Do Not Grow
#22	14	+	3, 5	1, 2, 3, 6, 9	8	—	1, 2, 3, 4, 5, 6, 9	
	10	+	2, 3, 4, 5, 6, 9	1	10	+	2, 3, 4, 5, 6, 9	1
#28	20	+	3, 4, 5	1, 2, 6, 9	9	—	1, 2, 3, 4, 5, 6, 9	
					1	+	3, 4, 5	1, 2, 6, 9
	3	+	2, 3, 4, 5, 6, 9	1	2	+	2, 3, 4, 5, 6, 9	1
	1	—		1, 2, 3, 4, 5, 6, 9				

* + = immune to λ *hd*⁺ *imm*⁴³⁴. — = sensitive to λ *hd*⁺ *imm*⁴³⁴. All isolates tested were sensitive to λ *hd*⁺ *imm* ^{λ} .

presence of the *hd*_{*n*} mutational site. When λ *hd*_{*n*} *imm* ^{λ} can grow on W3350(λ *gal* + *imm*⁴³⁴), the *hd*_{*n*} site is present; when not, it is absent. Those *hd* sites that are absent are, by definition, in the *dg* region of the particular syngenote tested.

Our results are as follows. If we start with one lysogenic syngenote, all the defective syngenotes produced by transduction with the lysate obtained from it are identical. On the other hand, if we work with lysogenic syngenotes that originate from different primary transductional events, we frequently find differences. We have observed these types of λ *gal* particles:

1. *hd*₁, *hd*₃, *hd*₄, *hd*₅, *hd*₁₀ present; *hd*₂, *hd*₆, *hd*₉ absent
2. *hd*₁, *hd*₃, *hd*₅, *hd*₁₀ present; *hd*₂, *hd*₄, *hd*₆, *hd*₉ absent

sometime before the isolation of the primary heterogenotes, and rarely if ever changes in the course of subsequent replications.

Table 3 shows the results of infecting W3350 with lysates from two different primary heterogenotes made from the phage λ *hd*₁ *imm*⁴³⁴. In this case, no lysogenic heterogenotes are produced, because W3350(λ *hd*₁ *imm*⁴³⁴) is itself a defective lysogen. The two types W3350(λ *gal* + *imm*⁴³⁴) and W3350(λ *hd*₁ *imm*⁴³⁴) (λ *gal* + *imm*⁴³⁴) are distinguishable, however, by the fact that the latter is sensitive to phages carrying *hd* markers in the *dg* region. The correctness of this interpretation is verified by testing galactose-negative segregants from both types. Those assumed to be W3350(λ *hd*₁ *imm*⁴³⁴) (λ

gal+ *imm*⁴³⁴) should give rise to galactose-negative segregants that are W3350(λ *hd*₁ *imm*⁴³⁴). These are immune, and all the mutants except λ *hd*₁ will grow on them. Heterogenotes of type W3350(λ *gal*+ *imm*⁴³⁴), on the other hand, are expected to produce mostly phage-sensitive segregants and a few homogenotes.

Segregation Patterns of Heterogenotes

The fact that defective syngenotes lose simultaneously their immunity to super-

We have also synthesized (a) double lysogens, carrying two defective (transducing) prophages W3350(λ *gal-1 gal-2*+ *imm* ^{λ})(λ *gal-1*+ *gal-2 imm*⁴³⁴), by transduction of W3350 with a mixture of lysates from two homogenotes, and (b) triple lysogens carrying one transducing and two active prophages. In both cases, we have observed frequent joint losses of more than one prophage element. Some of the data from lysogens of the first type are given in table 4. The number of sensitive segre-

TABLE 4. Segregation from Heterogenotes of the Presumed Structure W3350(λ *gal-1 gal-2*+ *imm* ^{λ})(λ *gal-1*+ *gal-2 imm*⁴³⁴)

Each galactose-negative strain was derived from a different galactose-positive colony, which was immune to λ and to 434. Of 136 galactose-positive colonies picked from the same plates, 129 were immune to both λ and 434, 6 were immune only to λ , and 1 was immune only to 434.

Type of Galactose-Negative Segregant		Heterogenote					Total
Immunity	<i>gal</i> + alleles present	91-3	91-5	91-7	39-5	39-7	
λ and 434	<i>gal-1</i> +	2	4	2	0	0	8
	<i>gal-2</i> +	0	0	2	2	0	4
λ	<i>gal-1</i> +	0	0	0	1	0	1
	<i>gal-2</i> +	6	6	3	9	4	28
	Neither	1	1	0	0	0	2
434	<i>gal-1</i> +	4	10	8	0	5	27
	<i>gal-2</i> +	8	6	6	0	2	22
	Neither	2	2	0	0	0	4
Neither	<i>gal-1</i> +	1	2	2	0	1	5
	<i>gal-2</i> +	4	3	4	3	3	17
	Neither	11	9	7	1	2	30

infection and one copy of the *gal* region constitutes the best evidence that the λ *gal* genome is a stable structure rather than a loose association between bacterial genes and phage genes. We have studied the segregation patterns of syngenotes produced in transduction experiments made with lysogenic recipients. The heterogenotes thus obtained are double lysogens, which show a correlated loss of the *gal*+ character and one set of prophage alleles. Furthermore, the alleles lost tend to be those that entered the cell at the time of transduction.

gants is far greater than would be expected on the basis of two independent losses within the same cell line.

There does not yet exist an adequate background of control data with nontransducing prophages to enable us to evaluate these situations in their proper perspective. All we can say is that the segregation patterns of the simpler types of heterogenotes strongly indicate the existence of a λ *gal* genome with genetic persistence. Those of the more complicated types provide little information about this question at the moment.

BACTERIAL GENETICS

M. Demerec, E. L. Lahr, T. Miyake, I. Goldman, E. Balbinder, S. Banić, K. Hashimoto, E. V. Glanville, and J. D. Gross

The members of our group studied a variety of problems concerned with the genetic structure of bacterial chromosomes, applying transduction and transformation techniques in experiments with *Salmonella typhimurium* and *Escherichia coli*. In addition to the workers named above, we had with us during a part of the year Dr. Pierre Fredericq, professor in the Department of Microbiology and Hygiene, University of Liège, who was a Fellow of the Belgian American Educational Foundation. Mrs. Jean W. McIntyre continued in charge of washing and sterilization of glassware, and Mrs. Emmy M. Snyder in charge of preparation of culture media.

During the summer of 1958 Dr. Philip E. Hartman, of the Department of Biology, Johns Hopkins University, carried on here his studies of histidine-requiring mutants in *Salmonella*. Temporary assistants during the summer were Miss Grace Bert, of Goucher College; Miss Anna Chao, of Bryn Mawr College; and Mr. W. Dilworth Cannon, of Yale University. Miss Elaine Staber, of Bennington College, worked as a temporary assistant from January 6 through March 7.

Our work received partial support from a grant-in-aid from the American Cancer Society.

Genetic Recombination by Transduction

During the past year Demerec, Goldman, and Lahr have conducted an extensive series of transduction experiments to study the occurrence of recombination among markers representing five linked loci (*tryD*, *tryC*, *tryB*, *tryA*, and *cysB*) and to interpret the findings in terms of a mechanism that would account for them. Previous studies have shown that the order of these five loci on a linkage map is that in which they are listed above. Thus, they divide into six regions a portion of bacterial chromosome that may be in-

cluded in one transducing fragment. In transduction, each recombinant colony originates from a bacterium in which part or parts of the genome have been replaced by genetic material from the homologous parts of a transducing fragment. The limits of such replacements, when they comprise portions of two or more of our marked regions, can be determined by analyzing the genetic constitution of the colony and identifying the marker or markers that are present. Since the end result of transduction is similar to the end result of crossing over, we employ the term "crossover" in discussing the exchanges of genetic material observed in transduction experiments.

Data were collected in two large experiments. In one, *tryD-10 tryB-4* bacteria were transduced by phage grown on *tryC-3 tryA-8 cysB-12* bacteria, and in the other *tryC-3 tryA-8 cysB-12* cells were transduced by phage grown on *tryC-10 tryB-4*. Out of 31 possible classes of recombination among the 5 markers, 26 were detected. The remaining 5, involving double crossovers in regions 2-4, 2-5, 3-4, and 4-5, include combinations of mutant markers that could not be recovered in our experiments. Since recombinants representing crossovers in these regions were found among the quadruple-crossover classes, there is no reason to doubt that those undetected classes do occur, and thus that all possible recombinations among the 5 markers take place.

The data of the two experiments show that under suitable experimental conditions recombinants representing double, quadruple, and sextuple crossovers are readily found. This may mean one of two things: either that the frequency of occurrence of crossing over is very high, or that the method of detecting recombinants is extremely sensitive. Analysis of the data leads us to think that frequency of

crossing over, and particularly of multiple crossing over, is very high in this short region of bacterial chromosome.

There was a considerable discrepancy between the values obtained in the two reciprocal experiments for the same quadruple-crossover classes. The discrepancy suggested that the incidence of crossing over may be influenced by the genetic constitution of the chromosome regions taking part in the event. To investigate this possibility, experiments involving three markers were carried out, since the results of the five-point tests seemed too complex. These experiments were designed so that one marker, of the three present in a test, would be selected by the medium on which the material was grown. Either *tryC-3* (abbreviation *C*) or *tryB-4* (*B*) was employed as the selected marker, and *tryA-8* (*A*) and *cysB-12* (*cys*) as unselected markers. In every experiment the recipient bacteria carried the mutant allele of the selected marker and either allele of the other two markers. The following six combinations were tested: *C A cys* (\times) +, *C* (\times) *A cys*, *C A* (\times) *cys*, *B A* (\times) *cys*, *B* (\times) *A cys*, and *B cys* (\times) *A*. The results show that recombinant classes carrying the wild-type allele of *tryA-8* or *cysB-12*, or both, were recovered with higher frequencies than recombinant classes carrying the mutant alleles of these two markers.

This difference in favor of wild-type alleles might be attributed either to a higher frequency of occurrence of crossing over when a wild-type allele was to be included in the recombinants or to a higher percentage of survival in a recombinant class carrying the wild-type allele. Reconstruction experiments with these same mutant strains did not disclose any selective disadvantage in growth or survival of either *tryA-8* or *cysB-12* when in competition with its wild-type allele. Therefore the first of the two possibilities mentioned seems more probable.

Our collection of tryptophan-requiring

mutants includes six alleles of the *tryA* locus. This made it possible to test the generality of the behavior observed in the previous experiment, that is, to find out whether the preference for inclusion of the wild-type allele rather than mutant alleles of *tryA* in recombinants was general or applied only in the case of certain alleles. The tests revealed a preference in favor of the wild type in the case of three of the alleles besides *tryA-8* (*tryA-47*, *-50*, *-52*) and none in the case of the remaining two (*tryA-24* and *-25*). Therefore it appears that mutant alleles of *tryA* differ with regard to preferential inclusion in a recombinant genome when in competition with the wild-type allele.

The mechanism responsible for the formation of recombinants in transduction experiments can be visualized in terms of a copy-choice model, which is a modification of the model proposed by Belling to explain the occurrence of crossing over. According to this concept, recombination occurs during chromosome replication. At the stage of cell division, it is assumed, a transducing fragment closely synapses with the homologous region of chromosome of the recipient bacterium, so that the region corresponding to the fragment is present in "duplicate." According to the model, chromosome replication takes place by the formation of replicas of small parts of the chromosome and the joining of these replicas in a zipper-like manner, starting from a certain point or end and proceeding toward some other point or end. As far as our material is concerned, the zipper action might proceed in either direction. In the unduplicated regions, only the chromosome of the recipient bacterium is available to furnish templates for the formation of replicas; but in the synapsed region, replicas may be modeled on either the recipient chromosome or the transducing fragment. Once a choice has been made, that is, after the first replica in the synapsed region has been formed, with either the fragment or the chromosome as a model, the copy

choices for subsequent replicas are not made at random. Although the next choice may be in either of the two synapsed strands, there is a higher probability that it will involve the same one than that it will switch to the other. If this were not so—if the chances for the two possible choices were equal—random segregation of markers (rather than linked transfer) presumably would result.

Our data indicate that the frequency with which switchovers occur depends on the genetic constitution of the chromosome regions involved. In the material tested, frequency of switchovers was higher when the opposite strand carried the wild-type allele of the *tryA* or the *cysB* locus and the choice was between it and the mutant allele *tryA-8*, *-47*, *-50*, or *-52*, or *cysB-12*. The frequency of switchovers was not significantly affected, however, when the choice was between the *tryA* wild-type allele and either *tryA-24* or *tryA-25*.

If a copy-choice mechanism is responsible for recombination, differences in preference for genetically different markers may be due either to variations in the degree of synapsis or to some other factor influencing the formation of replicas. The data available at present do not contribute any evidence that would enable us to distinguish between these two possibilities.

It is evident from our data that the replacement of portions of chromosome of a recipient bacterium by homologous portions of a transducing fragment is very frequent, much more so than the crossover exchanges observed in higher organisms. This may mean either that the opportunity for occurrence of such exchange is greater in transduction, because of close synapsis between a recipient chromosome and a transducing fragment, or that different mechanisms are responsible for crossing over and for transduction. It does not seem likely that in higher organisms double-crossover exchanges, of the type observed in transduction, occur frequently within short regions of chromosome and

are never detected. Methods now available in work with *Drosophila* and fungi are sensitive enough to detect such exchanges within short regions if they should be the rule rather than exceptions. On the other hand, it seems improbable that two different mechanisms would operate to produce such similar effects. A more reasonable interpretation is that some factor such as closeness of synapsis accounts for the fact that multiple crossing over within short regions is frequent in bacteria but not observed in higher organisms.

Second-Round Transduction

Studies of the kinetics of the transduction process, made by E. M. Witkin during the early period of our work on this problem (Year Book 53, pp. 241–244; Year Book 54, pp. 234–235), revealed that some of the offspring of a bacterial cell that has undergone transduction carry the intact genome of the recipient bacterium, whereas the rest carry a chromosome in which parts of this genome have been replaced by homologous parts belonging to the fragment genome (complete transduction). From the proportions of progeny cells carrying each kind of chromosome it can be concluded that complete transduction is accomplished during an early division of a cell containing a fragment.

Work done by E. S. Lennox with *E. coli* had demonstrated that complete transduction may occur again within the clone arising from a transduced cell. Studies carried on by Glanville during the past year have shown that such “second-round” transduction takes place also in *S. typhimurium*. Using the linked markers *tryB-10*, *tryA-8*, and *cysB-12* in one set of experiments, and several alleles of *leuA* along with several *ara* markers in another set, he detected transduction clones containing two classes of recombinant cells (“mixed colonies”). For example, experiment *leuA-39* (×) *ara-7* produced some clones made up of *leuA-39* + *ara-7* + cells and *leuA-39* + *ara-7* cells. The proportion

of such mixed colonies was low; they constituted 2 to 5 per cent of the total yield from complete transductions. The frequencies of various combinations of markers found in mixed colonies suggested that the original transducing fragment participates in a second round of transduction, which may occur either in a cell carrying the genome of the recipient bacterium or in a cell carrying a recombinant chromosome produced in first-round transduction. These data support the hypothesis, outlined earlier in this report, postulating that in the formation of a new chromosome the transducing fragment serves only as a source of templates.

Proline Mutants

In a continuation of work begun four years ago by Dr. D. Kanazir (see Year Book 54), genetic and biochemical studies of 168 proline-requiring mutants have been carried out by Miyake. Making abortive-transduction tests as a means of determining complementary relationships, he identified four gene loci and classified 120 of the mutants as follows: *proA* locus, 40 mutants; *proB*, 55 mutants; *proC*, 18; *proD*, 4; and *proAB*, 3. The remaining 48 mutants have too high frequencies of spontaneous reversion to be tested by this method.

Two of the four loci, *proC* and *proD*, are independent of each other and of the other two. The *proA* and *proB* loci, however, are closely linked and can be carried by one transducing fragment. This linkage relation was indicated by the results of experiments scoring frequency of occurrence of complete transduction. Numbers of transductants were much smaller in tests between markers of the *proA* and *proB* loci than in combinations involving either *proC* or *proD*. Further confirmation and conclusive evidence of close linkage between *proA* and *proB* were furnished by experiments with the multisite mutant *pro-47*, which covers all the known sites of both *proA* and *proB*.

The pathway of proline biosynthesis had been studied in *Neurospora* and *E. coli* by H. J. Vogel and several other investigators. Their work indicated that the sequence is as follows: glutamic acid—glutamic γ -semialdehyde— Δ^1 -pyrroline-5-carboxylic acid—proline. Tests by Miyake of the growth requirements of our proline mutants showed that synthesis in *proA* and *proB* mutants is blocked between glutamic acid and glutamic γ -semialdehyde, and that the block in *proC* mutants is between glutamic γ -semialdehyde and proline. Feeding tests demonstrated that *proC* mutants are able to feed *proA* and *proB* mutants. It was found that the growth requirements of two *proC* mutants (*proC-4* and *-124*) can be partly satisfied by either hydroxy-L-proline, glutamic acid, glutamic γ -semialdehyde, or proline. These mutants are tentatively retained in the *proC* group, however, since they behave like other *proC* mutants in complementation tests and feeding tests. At present it seems likely that leakiness is responsible for their exceptional growth requirements.

The position of the biosynthetic block in *proD* mutants has not yet been determined. Because these mutants grow slowly on proline-supplemented medium, and even on nutrient medium, their temperature sensitivity was investigated, as well as the possibility of another growth requirement; but no positive results were obtained. The *proD* mutants do not feed mutants of any other proline group. They show some growth on glutamic acid or glutamic γ -semialdehyde.

None of the proline mutants is able to grow on ornithine, and only *proC-4* and *proC-124* show slow growth on hydroxy-L-proline.

Among the 168 proline requirers studied, 7 are multisite mutants. No spontaneous reversion was detected in any of these, nor was it possible to induce reversion by treatment with ultraviolet radiation. Probably these mutants are the result of some

chromosomal aberration. One of them (*pro-47*) covers all the known sites of the *proA* and *B* loci, and also gives rise to very few transductants when acting as recipient with the wild type as donor, from which we may infer that the aberration extends over the section of chromosome comprised in a transducing fragment, so as to leave little room for crossing over to occur. Mutant *pro-21* covers all the known sites of locus *proB* and some sites of locus *proA*, whereas *pro-126* covers some sites of the *proA* and *B* loci. Two of the multisite mutants cover a few sites of *proA*, and two others a few sites of *proB* and *proC*, respectively. Tests with 9 *proA*, 16 *proB*, and 3 *proC* mutants showed that all are different; in this material, evidently, repeated changes at the same mutational site are not common.

Leucine, Isoleucine, Valine, Threonine, Aspartic Acid, and Glycine Mutants

Biochemical studies made in several laboratories have indicated that the pathway of isoleucine biosynthesis is through L-aspartate, L-homoserine, L-threonine, α -ketobutyric acid, acetohydroxybutyric acid, dihydroxyisoleucine, and ketoisoleucine, to isoleucine. Valine originates from pyruvate through the dihydroxy and keto acids. Ketovaline gives rise to ketoleucine, which is converted to leucine. Although the precursors are different, the same enzymes are involved in the last steps of isoleucine and valine synthesis, so that a single mutation may bring about a double requirement.

Our collection includes about 170 mutants that require certain of the amino acids just enumerated. During the past year Glanville has investigated the relations among these mutants by observing their behavior with respect to complete and abortive transduction. Relative frequencies of complete transduction indicate whether or not mutants are closely enough linked to be carried in one transducing fragment; occurrence of abortive trans-

duction reveals complementation between mutants. In the case of closely linked, phenotypically similar mutants, observations of abortive transduction help in analyzing allelic relationships and thus in determining whether particular mutants are due to changes at one gene locus or at several closely linked loci. Abortive transduction cannot be detected in some groups of mutants; and, unfortunately, leucine-requiring mutants, of which we have 105, are in this category.

The following symbols designate the various groups of mutants investigated:

- asa*—require aspartic acid
- gly*—require glycine but not serine
- ile*—require isoleucine
- ilva*—require isoleucine and valine
- ilvl*—require isoleucine, valine, and leucine
- leu*—require leucine or ketoleucine
- thr*—require threonine

According to transduction tests, the 105 *leu* mutants form a single linkage group. Although several gene loci are presumably involved in the conversion of ketovaline to ketoleucine, they could not be identified in our material, since no method of detecting abortive transduction could be worked out.

Fourteen *ilva* mutants form one linkage group, which is subdivided by abortive-transduction tests into three groups, as follows: *ilvaA*, 5 mutants; *ilvaB*, 7; and *ilvaC*, 2. Complementation is observed between members of different groups but not within the same group. The *ilvaC* mutants grow on the dihydroxy acids, whereas *ilvaA* and *ilvaB* do not.

Eleven *ile* mutants form a single linkage group, within which there is no complementation. Growth of *ile* mutants can be supported by either α -ketobutyric acid or α -aminobutyric acid.

Thirty-two *thr* mutants, all linked, are divided by nutritional requirements into two groups. Each of these groups is further subdivided by complementation tests. Members of the *thrA* group (14 mutants)

and *thrB* group (14 mutants) require L-threonine for growth, but *thrC* (3) and *thrD* (1) will grow slowly on homoserine as well as rapidly on L-threonine. Growth of *thrC* and *D* on homoserine is accelerated by the addition of isoleucine.

One mutant is known, *val-1*, which grows slowly on valine or ketovaline. Growth is greatly stimulated by the addition of isoleucine or ketoisoleucine.

On the basis of both linkage and complementation tests, 2 *asa* mutants belong to a single linkage group. The same is true of 3 *gly* mutants (*glyA*).

Linkage studies. By means of the transduction technique, it is possible to detect linkage between markers that are close enough together to be carried in the same transducing fragment. In work with *S. typhimurium* and phage PLT-22 these fragments are short, and therefore only close linkage can be detected. Even so, it may not always be possible to discover linkage between two adjacent markers, if transducing fragments result from breaks at predetermined points in the bacterial chromosome, as was indicated by studies made by Ozeki (see Year Book 56).

Nevertheless, the best evidence of linkage between two loci is the observation that they can be simultaneously transduced. In the case of phenotypically similar mutants, when a donor class cannot be obtained, a low frequency of recombinants is satisfactory evidence of linkage. When three or more loci are found to be linked, their order on a linkage map can be unequivocally determined by three-point tests such as those made to ascertain the order of four tryptophan loci and the *cysB* locus (see Carnegie Inst. Wash. Publ. 612). Linkage order can also be approximately determined by observing frequencies of recombination between the linked markers in transduction tests. Since it is known that various factors may affect frequency of observed recombinants, however, this means of determination is not reliable.

Low frequencies of recombination be-

tween members of the three *ilva* groups indicated that the loci are linked. Linkage was confirmed by the finding that when *ilvaA* or *ilvaB* mutants are infected with phage from *ilvaC* bacteria, and grown on medium containing the keto acids, some of the recombinants, like the donor, require the keto acids for growth.

Tests of simultaneous transduction indicate that the *ileA* locus is linked to the *ilva* loci but not to any of the *thr* loci. The *val-1* mutant shows linkage with *ile* and *ilva*. The relative positions of the five loci involved in this linkage system cannot be definitely determined without the application of three-point tests; but two-point tests indicate that the order is *ileA-ilvaA-ilvaB-ilvaC*, with the position of *val* uncertain but probably close to *ilvaC*.

When *serB* bacteria are infected with phage from *thrA*, *B*, *C*, or *D* cells and plated on medium supplemented with threonine, a characteristic proportion of the transductants require threonine. The frequencies of introduction of the threonine requirement when *serB-10* is transduced with phage grown on *thrA-10*, *B-12*, *C-11*, and *D-16* are 12, 18, 28, and 31 per cent, respectively, which indicates that the order of the five loci on the chromosome is *thrA-thrB-thrC-thrD-serB*.

The *leuA* locus is linked to the arabinose loci *araA* and *araB*, which are distinguished on biochemical grounds by the fact that *araB* mutants accumulate ketopentose whereas *araA* mutants do not. Three-point tests have shown that the linkage order is *leuA-araA-araB*.

No linkage has been detected between the *thr* loci and *asaA* or between the *thr* loci and any of the methionine loci.

In summary, the four loci controlling threonine synthesis are closely linked with one another and also with a serine locus *serB*. The five loci controlling synthesis of the biochemically closely related compounds isoleucine and valine form a cluster on the chromosome. The leucine locus *leuA* has shown linkage only to *araA* and *araB*.

Transduction of Colicinogenicity

Transduction in S. typhimurium. FredERICQ studied transduction by infecting the noncolicinogenic strain LT-2 of *S. typhimurium* with phage PLT-22 grown on LT-2 derivatives made colicinogenic by mixed cultures with colicinogenic *E. coli* or *Shigella sonnei*.

The ability to transduce prototrophic markers or resistance to streptomycin was the same whether the phage was grown on a colicinogenic or a noncolicinogenic strain. In both cases the transductants were noncolicinogenic when the recipient strain was noncolicinogenic, and kept the original ability to produce colicin when the recipient strain was colicinogenic.

Selection for transfer of colicinogenicity was made in the following manner. Serial tenfold dilutions of the infected bacteria were plated on ordinary nutrient agar and immediately covered by a top layer of soft agar. Cells drawn to the surface were killed by chloroform vapors to prevent the occurrence of surface colonies. After 48 hours of incubation at 37° C the surface of the agar was inoculated with *E. coli* strain B, to serve as an indicator of colicin production. After a further 24 hours of incubation the plates were examined for the presence of inhibition zones in the confluent growth of the indicator.

This technique is essentially the same as that previously used to demonstrate colicinogenic transfer in mixed cultures. The frequency of transfer by phage is much lower, however, and inhibition zones were seen only on crowded plates, where the transduced colicinogenic cells were completely obscured by the noncolicinogenic bacteria. To isolate these transduced cells, the entire growth underlying an inhibition zone was punched out with glass tubing and suspended in broth. The suspension, which contained a large proportion of transduced cells, was serially diluted and plated, in a further selection for colicin production. After one or more such cycles of enrichment, plates were obtained on

which inhibition zones contained well isolated colonies. These colonies were picked by stabbing through the agar, and their colicinogenic ability was verified after a further isolation on agar. Inhibition zones could be seen on plates containing 1×10^5 to 1×10^6 colonies; but on plates inoculated with a larger number of bacteria the indicator *E. coli* strain did not develop, probably because of a staling effect of the *Salmonella* culture.

Transduction of the colicinogenic property was repeatedly observed with two phage preparations grown on two independently obtained colicinogenic LT-2 cultures, producing colicin E(2). The transductants had all the markers of the recipient strains, and received no markers except colicinogenicity from the strains on which the phage was grown. The frequency of colicinogenic transduction was of the same order of magnitude as that of prototrophic transduction. When a fully grown aerated broth culture was infected with a multiplicity of 10, the ratio of colicinogenic cells was approximately 1 per 10^6 .

Salmonella made colicinogenic for colicins other than E(2) was also tested, but without success. One strain, which produced colicin K, had lost the susceptibility to phage PLT-22 and could not be utilized to grow the phage. Another produced colicin I, which is not a very potent colicin; it is doubtful whether inhibition zones due to that colicin could have been detected on crowded plates.

Transduction in E. coli. Noncolicinogenic auxotrophs derived from *E. coli* strain K-12 were infected with phage P1 grown on colicinogenic derivatives of K-12, obtained by mixed culture with different colicinogenic strains. The techniques employed in selecting for prototrophy or for colicin production were essentially the same as in the *Salmonella* experiments. The colicinogenic strains on which the P1 phage was grown included strains producing colicins E(1), E(2), V, B, K, and I.

The ability to transduce prototrophic markers was much reduced, and in several cases completely abolished, when the phage was grown on a colicinogenic strain. This inhibition could have been due to a killing effect of free colicin present in the phage preparations; but the same effect was also observed when the phage preparations had no demonstrable colicin activity or when they were acting on bacteria resistant to the colicin.

When selection was made for colicinogenicity, colicin transduction was repeatedly obtained with two different phage preparations, one grown on a strain producing colicin E(1) and the other on a strain producing colicin B. In both cases the rate of transfer was about 1 per 10^6 surviving bacteria when the multiplicity of infection was between 5 and 10. Transfer of colicin E(2), carried out successfully in *Salmonella*, could not be achieved in *E. coli*.

Transduction to colicinogenicity seems to be independent of the transfer of other markers. Colicinogenic transductants do not receive markers other than colicinogenicity. Furthermore, the ability of a phage preparation to transfer colicinogenicity is not correlated with its ability to transfer prototrophic markers. For example, the phage grown on a strain producing colicin E(1) produced about 10 times more prototrophic than colicinogenic recombinants (the rate of transduction to prototrophy with this phage was still 10 times lower than that with phage grown on the control noncolicinogenic strain). On the other hand, the phage grown on a strain producing colicin B produced about the same number of colicinogenic transductants as the E(1) phage, but no prototrophic transductants. This difference may have been due to a killing effect of the colicinogenic factors, for it had previously been observed in recombination experiments that colicinogenic transfer is often lethal.

Mutability Factor

The two strains of *S. typhimurium* used in our laboratory differ strikingly in their mutability patterns. Strain LT-7 is considerably more mutable than LT-2, and auxotrophic mutants are much more easily found in LT-7 cultures. Moreover, a higher proportion of the auxotrophs isolated in LT-7 are very mutable; they revert with a frequency 100 to 1000 times higher than that of the auxotrophs usually found in strain LT-2. Since auxotrophs having an intermediate range of mutability are rare, classification into types showing high and low frequencies of reversion can readily be made. Highly revertible types have been found among the alleles of all the loci studied—proof that such mutations are not limited to any one locus, and an indication that they probably are not restricted to any one group of loci.

It has been found that our LT-7 strain is heterogeneous with respect to presence or absence of a factor (very likely a gene) that is responsible for the characteristic mutability pattern of the strain. Among lines derived through single-colony isolation from LT-7, some exhibit the mutability pattern of LT-7 and some the mutability pattern characteristic of LT-2.

Experiments are in progress by Miyake to analyze the nature of the factor responsible for the more typical high-mutability pattern of strain LT-7. It appears very probable that this factor is a gene, similar to mutator genes found in *E. coli* and in several other organisms. The evidence now available, however, indicates that the factor increases mutation rate in both directions—from wild type to auxotroph and from auxotroph back to wild type—and also that its effect is fairly general, since it has been detected in relation to at least ten gene loci.

Alleles of the same gene locus very commonly differ from one another in mutability pattern. In our work with auxotrophs, we have found characteristic differences in frequency of spontaneous rever-

sion as well as in frequency of reversion induced by treatment with different mutagens. A particularly striking kind of variation in mutability pattern results in what we call mutagen-stable types. These mutate spontaneously, thus demonstrating a capacity for mutation; but the degree of their mutability cannot be increased by treatment with mutagens.

Studies made in our laboratory have contributed a considerable amount of data about mutagen-stable and mutagen-labile auxotrophs. Comparative studies of mutability carried out several years ago with 35 auxotrophs isolated in strain B of *Escherichia coli* revealed that 5 of them, or 14.3 per cent, were mutagen stable. These 5 were tested with ultraviolet radiation, X rays, and several potent chemical mutagens (nitrogen mustard, manganous chloride, 1:2:3:4-diepoxybutane, and 2:4:6-tri(ethyleneimino)-1:3:5-triazine); and mutations, although they occurred spontaneously, could not be induced by any of the treatments. A considerably larger proportion of auxotrophs isolated from strains LT-2 and LT-7 of *Salmonella typhimurium* was also found to be mutagen stable. Several of these auxotrophs were tested with all the potent mutagens we had available; but, because the preliminary results indicated that an auxotroph that did not respond to UV treatment was also not susceptible to treatment with any of the other mutagens, subsequent tests were made with UV only. Therefore, most of the findings regarding mutagen stability of LT-2 and LT-7 auxotrophs apply to UV stability in particular, although they are probably true for other mutagens as well.

In the *Salmonella* material, tests were completed with 67 *his*, 80 *pro*, and 16 *ser* auxotrophs—163 in all. In 93 of these, or 57.1 per cent, reverse mutations were not induced by UV treatment. The 163 auxotrophs tested included all those selected in strains LT-2 and LT-7 and in their derivatives from recombination experiments, except those which revert spontaneously

with such high frequencies that induced reversions could not have been detected.

The analyses of inducibility of reversions in auxotrophs revealed two interesting features. They showed that in the *Salmonella* material the frequency of mutagen-stable auxotrophic mutants is very high, almost 60 per cent. They also demonstrated clearly that different genera of bacteria may differ greatly in this regard, for among the 35 *E. coli* auxotrophs tested only 14.3 per cent were mutagen stable, whereas among the 163 *S. typhimurium* auxotrophs there were 57.1 per cent.

The 5 mutagen-stable *E. coli* auxotrophs are tryptophan-, histidine-, proline-, and methionine-requiring mutants. In *S. typhimurium*, mutagen-stable types have been found in all the mutant groups of which appropriate tests have been made, namely, the histidine, proline, serine, tryptophan, cystine, purine, and galactose groups. Therefore it appears likely that mutagen-stable types are not specific to certain groups of mutants, but occur generally.

In *Salmonella*, these types have appeared frequently enough to enable us to obtain data regarding their distribution among certain groups of mutants and among alleles of certain gene loci. The data show that observed frequency of mutagen-stable types was similar among three groups of mutants (histidine, proline, and serine), and also that mutagen stability was found among alleles of all seven *his* and all four *pro* loci studied.

Thus the available experimental evidence shows that the alleles of a gene locus that can be traced to changes at single mutational sites may be either mutagen stable or mutagen labile, and that in *S. typhimurium* most of them are mutagen stable. It appears probable that within a locus the sites representing mutagen-stable and mutagen-labile alleles are not grouped separately but arranged in mixed order.

Reversions among Tryptophan Mutants

In mutability studies with 13 tryptophan-requiring auxotrophs, Balbinder found

that certain of them produced not only the expected large-colony revertants but also some small colonies. These were similar in appearance to the "revertant" colonies observed with adenine-requiring mutants by Yura (Year Book 54) and with cystine-requiring mutants by Howarth (Year Book 56), which proved in each case, when analyzed by a method developed by Yura, to be due to suppressor mutations.

The genetic constitution of the tryptophan small-colony mutants was analyzed by Yura's method and by linkage tests. The results showed that the small-colony character persists in broth transfers; that it is the result of a genetic change, since it can be transferred to other strains in transduction experiments; and that this change is at a suppressor locus, which is not closely linked with the tryptophan locus involved, *tryD*.

Suppressor mutants isolated from *tryD-6*, *-7*, and *-10* were tested with *tryD-1*, *-6*, *-7*, *-9*, *-10*, and *-11*. All these alleles except *tryD-10* and *-11* recombine with one another; *tryD-10* and *-11* recombine with all the others, though not with each other. Five of them revert spontaneously; but in the case of the sixth (*tryD-11*) only three apparent revertants were found among about 10^{11} bacteria, and possibly these three prototrophs were contaminants. Since, also, we have not been able to induce reversions in *tryD-11* by ultraviolet treatment, it appears very likely that this mutant is the result of a chromosomal aberration, probably a small deletion. Results of the tests showed that the suppressor mutations are specific; each of them acted upon only the *tryD* allele from which it had been isolated. The suppressor of *tryD-10* did not affect *tryD-11*.

Small-colony mutants were also readily observed in *tryD-1*, *tryB-2*, *tryC-3*, and *tryA-8* cultures. It is assumed that these too were due to suppressor mutations, for all such small-colony "revertants" studied in our laboratory, although originating in

many different auxotrophs and analyzed by various workers, have proved to be suppressor mutants.

In a comparative study of spontaneous and ultraviolet-induced mutability, carried out with the 13 tryptophan mutants, frequencies of large-colony and small-colony revertants were recorded. Presumably, the large-colony revertants were due to true reversions at the sites of the original mutations, and the small-colony "revertants" were due to suppressor mutations. The results of some tests are given in table 5.

TABLE 5. Mutability, Spontaneous (S) and Ultraviolet-Induced (UV), in a Series of Tryptophan Auxotrophs of *Salmonella typhimurium*, with Reference to Reversions to Prototrophy and Mutations at a Specific Suppressor Locus

Values represent numbers of mutations per 10^9 surviving cells. UV radiation, 620 ergs/mm².

Mutant	Reversion		Suppressor Mutation	
	S	UV	S	UV
<i>tryD-1</i>	0.5	1680	?	10,040
<i>tryD-7</i>	0.8	0	2.1	138,750
<i>tryD-11</i>	?	0	0	0
<i>tryD-10</i>	0.2	7	0.1	815.5
<i>tryD-6</i>	0.7	175	0.1	90.8
<i>tryD-9</i>	0.2	0	0	0
<i>tryB-2</i>	0.3	5	0.2	4.0
<i>tryB-4</i>	1.4	564	?	?
<i>tryB-19</i>	1.5	5235	0.6	0
<i>tryC-3</i>	1.4	4340	?	56.5
<i>tryA-8</i>	2.8	1856	1.1	2,380

They demonstrate once again the specificity of mutants with regard to spontaneous and induced mutability, and provide additional evidence that this specificity resides in the allelic site, not in the gene locus. In *tryD-6*, for example, both reversions and suppressor mutations were induced with about equal frequency; in *tryD-1*, suppressor mutants were induced considerably more often than revertants; and in *tryD-7* no reversions were induced whereas the rate of induction of suppressor mutations was very high.

Transduction Technique for E. coli

Phage P1 was originally isolated from *E. coli* strain Lisbonne-Carrère by G. Bertani, and first used in transduction experiments by E. S. Lennox, who employed a mutant adapted to grow on *E. coli* strain K-12. More recently Skaar and Davidson, working with a strain (P1b) derived from that of Lennox, which plates fairly well on *E. coli* B/r, studied the structure of the tryptophan region of that bacterium by the transduction method. During the past year Hashimoto and Gross have made further improvements in technique for transduction experiments with strain B/r and P1 phage.

When P1b was assayed by Hashimoto on *E. coli* B/r, plaques of two types in approximately equal proportions were observed, one turbid and the other relatively clear. The two types were purified, and stocks of each were made. The turbid-plaque former was designated P1bt and the clear-plaque former P1bc. Although both substrains are capable of bringing about transduction, P1bt was used exclusively in this work.

Phage stocks were prepared by a modification of the confluent lysis plate technique described by Swannstrom and Adams, with tris glucose medium, which gives optimal yields of about 10^{10} plaque-forming particles in this system. Transduction experiments were carried out as with *Salmonella*, except that calcium was added to the adsorption tube to a final concentration of 2.5×10^{-3} M and adsorption was allowed to proceed for 20 to 30 minutes, by which time at least 80 per cent of the phage had adsorbed. The frequency of transduction per plaque-forming particle was between 10^{-4} and 10^{-5} for auxotrophic mutants when the multiplicity of infection was low, and somewhat less at high multiplicity. The frequency with which transduction of resistance to various drugs occurred was considerably lower, of the order of 1 to 5×10^{-7} per plaque-forming particle. In the case of resistance

to azide the observed low frequency has been shown to be due to unavoidable technical difficulties in the detection of transductants.

Transductions in E. coli

In work carried on by Gross an attempt has been made to investigate certain aspects of the *E. coli* B/r-P1 transduction system. Attention has been focused on the *thr ara leu azi-r pan T1,5-r* region (threonine requirement, inability to utilize L-arabinose, leucine requirement, azide resistance, pantothenate requirement, resistance to phages T1 and T5, with the loci in that order). This region of the bacterial chromosome is well known from studies of the process of sexual recombination in strain K-12, and has been investigated in some detail in transduction experiments by E. S. Lennox, also made with K-12.

Nonuniformity of transducing fragments. When a *thr leu pan* strain was infected with phage grown on wild-type B/r bacteria it was found that joint transduction of *thr* and *leu* and of *leu* and *pan* can occur with appreciable frequency, but that *thr* and *pan* cannot be transduced simultaneously. (The presence of the *pan+* marker in as few as 0.1 per cent of the *thr+* *leu+* colonies could have been detected.)

Other experiments have provided examples of the effect of selection for the joint transduction of two distantly linked markers on the simultaneous transduction of a third, which is closely linked to one of the selected markers. For example, *ara-1* shows 74.5 per cent joint transduction with *leu* on one side and 4 per cent with *thr* on the other. When selection was carried out for joint transduction of *thr* and *ara*, only 31.6 per cent of the transductants were *leu+*. Similarly, in an experiment in which *azi-s pan T1,5-r* recipients were transduced by phage grown on *azi-r pan+ T1,5-s* cells, the reduction in the proportion of *T1,5-s* transductants when *azi-r* and *pan+* were selected simul-

taneously was of the same order, namely, from 61 per cent to 23.1 per cent. With a *thr leu azi-s* recipient and *thr+ leu+ azi-r* donor, about 40 per cent of the *leu+* transductants were *azi-r* whereas only 1 *azi-r* was found among about 200 *thr+ leu+* transductants. The much larger reduction observed in this experiment is probably related to the fact that the distance between *thr* and *leu*, as estimated by frequency of joint transduction, is greater than the distance between the selected markers in the two previous examples.

It appears, therefore, that whenever selection is carried out for the simultaneous transduction of two distant markers there is a reduction in the proportion of transductants that carry any unselected marker not situated between the selected markers. The magnitude of this reduction increases with increased distance between the selected markers, or between the selected and unselected markers. In the extreme case, the proportion of transductants containing the unselected marker may drop to zero, as in the experiment with a *thr leu pan* recipient.

The most obvious interpretation of these results is that the fragments participating in transduction of a given marker are not uniform, but represent more or less random portions of the corresponding region of the chromosomes of the donor cells. The observations point to a restriction in size of the fragments, in that a fragment carrying any particular marker, and extending a considerable distance to one side of that marker, extends, on the average, a short distance to the other side.

An alternative interpretation is that the fragments are in fact uniform, and that the effect of selection is due to positive interference in the incorporation event. For example, in the experiment with *thr ara+ leu azi-s* as recipient and *thr+ ara+ leu+ azi-r* as donor, *thr leu+* transductants result from crossing over in region 2 or 3 and in region 4 or 5, whereas *thr+ leu+* transductants result from crossing over in re-

gion 1 and either 4 or 5. If there is positive interference, a higher proportion of the *thr leu+* class will have crossovers in region 5, and will therefore incorporate the *azi-r* marker.

To test this hypothesis the experiment was repeated with the recipient carrying *ara* in addition to its other markers. The *thr+ ara leu+* and *thr ara leu+* classes both involve crossovers in region 3 and should, according to the interference hypothesis, contain the same proportion of *azi-r* transductants, since there will be the same amount of interference in each case, whereas the *thr+ ara+ leu+* class, which involves a crossover in region 1 but not in region 3, should contain a much lower proportion. Contrary to this expectation, it was found that the *thr+ leu+* class as a whole, irrespective of *ara* genotype, contained no *azi-r* (among 50 transductants tested) whereas the *thr ara leu+* class included 40 per cent *azi-r*. It appears, therefore, that a uniform-fragment hypothesis cannot account for the effect of selection. It should be mentioned that 140 *thr+ leu+* and about 130 *thr leu+* transduction colonies were tested for impurity with regard to arabinose marker, and only 5 mixed colonies were found.

Recombination between closely linked sites. Recombination among three closely linked arabinose mutants (*ara-1*, *ara-2*, and *ara-3*) has been studied, and the results provide some further information about the properties of the transduction system. The mutants were isolated in a *thr leu* strain, and subsequently in each case the threonine and leucine requirements were removed by transduction. It was then possible to do experiments involving all combinations of *ara-1*, *ara-2*, *ara-3*, and *ara+*, with the recipient in each case *thr leu* and the donor *thr+ leu+*. When each mutant was transduced with phage grown on wild type, and *ara+* transductants were scored for *thr* and *leu*, it was found [table 6, section (a)] that most had been simultaneously transduced to *leu+* and

only a few were *thr*+. The *ara-2*+ transductants had the lowest proportion of *leu*+ and the highest of *thr*+, which suggests that the *ara-2* site is the nearest to *thr*. The order of the sites with respect to one another and to *thr* and *leu* can be determined unambiguously from the results of the reciprocal experiments, shown in

TABLE 6. Three- and Four-Point Tests Involving Nonidentical *ara* Mutants and the Markers *thr* and *leu*

In every experiment the recipient was *thr leu*, the donor *thr*+ *leu*+; only the *ara* marker varied, as indicated in the table under the headings "donor" and "recipient." After adsorption, mixtures of bacteria and phage were plated onto minimal medium supplemented with threonine and leucine (20 µg/ml of each) and containing L-arabinose (0.2 per cent) as sole carbon source. Only *ara*+ bacteria can grow on this medium. The transductants were tested for threonine and leucine requirements.

Recipient	Donor	Percentage of <i>ara</i> + Transductants Containing the Un- selected Marker	
		<i>thr</i> +	<i>leu</i> +
Section (a)			
<i>ara-1</i>	<i>ara</i> +	4.0	74.5
<i>ara-2</i>	<i>ara</i> +	5.2	59.6
<i>ara-3</i>	<i>ara</i> +	4.3	79.6
Section (b)			
<i>ara-1</i>	<i>ara-2</i>	1.2	69.9
<i>ara-2</i>	<i>ara-1</i>	7.4	17.6
<i>ara-1</i>	<i>ara-3</i>	6.4	20.5
<i>ara-3</i>	<i>ara-1</i>	2.4	52.6
<i>ara-2</i>	<i>ara-3</i>	9.5	13.9
<i>ara-3</i>	<i>ara-2</i>	2.8	70.3

table 6, section (b). One experiment in each pair gave a high proportion of *leu*+ and a low proportion of *thr*++; the other gave a much lower proportion of *leu*+ and about three times as many *thr*+ as the first experiment. It is inferred that in the former cases the *thr*+ *ara*+ class results from a quadruple crossover, and the *ara* site in the donor is nearer to *thr* than is the *ara* site in the recipient, whereas in the latter cases the reverse is true. These

results indicate that the order of the mutational sites is *thr-ara-2-ara-1-ara-3-leu*.

A comparison of sections (a) and (b) of table 6 yields two points of interest.

1. When *thr ara-2 leu* bacteria were transduced by phage grown on wild-type cells, 5.2 per cent of the *ara*+ transductants were *thr*++; but when the donor was *thr*+ *ara-1 leu*+ or *thr*+ *ara-3 leu*+, the proportion of *thr*+ transductants was significantly greater—7.4 per cent and 9.5 per cent, respectively. In both cases the *ara* site in the donor is farther from *thr* than the *ara* site in the recipient. A similar increase was observed with *thr ara-1 leu* as recipient and *thr*+ *ara-3 leu*+ as donor.

2. If the same kind of comparison is made with respect to the proportion of transductants carrying *leu*+, the result is entirely different. When the *ara* site in the donor was farther from *leu* than the site in the recipient, the proportion of *leu*+ transductants was lower than when the donor was wild type. For example, *thr ara-3 leu* gave 79.6 per cent *leu*+ with a wild-type donor but only 52.6 per cent with *thr*+ *ara-1 leu*+ and *thr*+ *ara-2 leu*+ donors. It may be noted that *ara-1* is closer to *ara-3* than *ara-2* is.

The first of these observations finds a ready explanation in the hypothesis proposed above concerning the nonuniform constitution of transducing fragments. The nature of the explanation may be seen by reference to figure 3. In an experiment with wild type as donor (*ara-3*+ instead of *ara-3* as in the diagram), a crossover anywhere in region 1 and in region 2 or 3 would produce *ara*+ transductants. The contributions of the two types of fragment, *a* and *b*, to the transductants formed would be equal, since their contribution is a function of the distance they extend on either side of the *ara-2* marker, and this distance is the same for the two types of fragment, though reversed with regard to region. In the experiment with donor carrying *ara-3*, on the other hand, one crossover *must* occur in region 2 and the other may occur anywhere in region 1.

The contribution of the two types of fragment will be independent of the distance they extend in region 3 and will depend only on the distance they extend in region 1. The fragments of type *a* will therefore contribute more, and there will be a higher proportion of *thr*⁺ in this experiment than in the experiment with wild-type donor. The same effect will, of course, be observed even if the number of fragment types is large and their size variable.

The second set of observations does not appear to be explicable in terms of non-uniform fragment constitution, and seems to indicate the presence of negative interference in the recombination process itself.

hours before streptomycin was added in another layer of agar. It was also found possible to transduce *str-d* mutants to sensitivity in 6 out of 15 cases tested. The 9 strains that failed to show transduction produce only very slight growth on medium not containing streptomycin, and it appears probable that the number of divisions they undergo on such medium is not adequate for the expression of transductants.

Experiments were made to determine whether mutants of independent origin are due to mutations at one or at several gene loci. Reciprocal transduction tests with 6 *str-d* mutants showed that only one gene locus is involved. Moreover, since

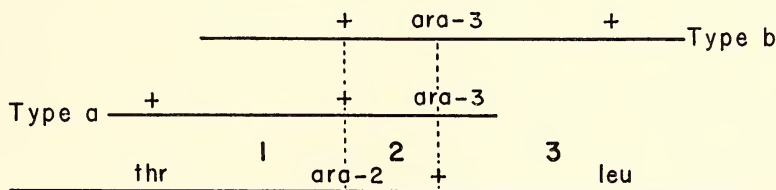


Fig. 3. Simplified diagram of an experiment with recipient *thr*⁺ *ara-2* *leu*⁻ and donor *thr*⁺ *ara-2*⁺ *ara-3* *leu*⁺. The fragments carrying the *ara-2*⁺ marker are considered for the sake of convenience to be of only two types, *a* and *b*, occurring with equal frequency. They are of the same length; type *a* extends a certain distance in region 1 while type *b* extends the same distance in regions 2 and 3. Only the type *a* fragments carry the *thr*⁺ marker.

The effect discussed above may also be present here, and would act in the opposite direction to the interference, but since *leu* is very close to *ara* it would be considerably weaker than the interference.

Streptomycin Resistance

Working with strain B/r of *E. coli* and phage P1, Hashimoto has studied genetic relations among streptomycin-dependent (*str-d*), -resistant (*str-r*), and -sensitive (*str-s*) mutants, and nondependent "revertants" from *str-d*. In experiments with 6 *str-d* and 2 *str-r* mutants he obtained evidence that both resistance and dependence can be transferred to the sensitive strain by transduction. To allow time for the expression of resistance in transduced cells, the recipient bacteria were inoculated into nutrient agar and incubated for 3

no recombinants were observed, the results indicate either that the sites of these mutations are very close together or, more probably, that they are multisite changes. It should be pointed out, however, that the technique for detecting recombinants in the streptomycin-resistance system is considerably less sensitive than the technique employed with auxotrophs.

Similar tests were carried out to determine whether or not different gene loci are responsible for dependent and resistant mutants. These tests involved one *str-d* mutant as recipient and *str-r-2* and *str-r-3* as donors. In the *str-r-2* experiments, 4 transductants, among approximately 4200, were wild type; and in the *str-r-3* experiments there were 3 wild-type transductants among about 7000. Since wild-type variants were not observed on the

control plates, it is assumed that the 7 found in the experimental series were recombinants. These results indicate that the changes responsible for *str-r* and *str-d* are either at the same gene locus or at two closely linked loci, and that at least some of the mutants included in the experiments may be multisite mutants.

It is well known that streptomycin-dependent bacteria may mutate to nondependent types, which are either sensitive or resistant to streptomycin. This mutability from dependence to nondependence was extensively utilized in our earlier studies of mutagenicity of various agents (see Year Books 48 and 49). The recent development of a technique for applying transduction to *E. coli* has made it possible to analyze such nondependent mutants genetically, that is, to determine whether "revertants" are due to back mutation at the *str-d* locus or to changes in some other part of the genome. Forty-two sensitive or slightly resistant mutants derived from strains *str-d-77* and *str-d-5* were analyzed by transduction tests in which they acted as recipients, with an *str-s* strain as donor.

In all cases it was found that the sensitive or slightly resistant mutants carried an *str-d* gene; in other words, they had not originated by reversion at the *str-d* locus but by an independent mutation at another locus, which suppressed the activity of *str-d*. Further tests with 16 of these suppressor mutants showed that they are all allelic; only one suppressor gene locus is involved. The suppressor mutations are not specific; it was found that any one of them affected the expression of all *str-d* and *str-r* genes tested. The effect on the *str-r* phenotype is to modify it from a high degree of resistance to a low degree.

The linkage relation between the suppressor gene and *str-d* was investigated with two suppressor mutants. The data indicate close linkage between the two genes.

The results of these studies as a whole reveal that the genetic control of high

streptomycin resistance and dependence in *E. coli* resides within a short region of chromosome, which also contains a genetic mechanism for control of the degree of resistance.

Penicillin and Chloramphenicol Resistance

Transduction techniques have been employed by Banič to study the genetics of multistep resistance to penicillin and to chloramphenicol in *S. typhimurium* strain LT-2. It appeared probable that such a study would reveal: (a) a polygenic system controlling sensitivity and resistance to these drugs, and (b) development of multiple resistance by mutation at different sites of the same gene locus.

Banič attempted to discover whether first-step penicillin or chloramphenicol resistance could be transferred to sensitive recipient strains by transduction; whether the full resistance of second-, third-, or fourth-step strains could be transferred to sensitive recipients; and whether multistep resistance could be built up by transduction.

Two methods were developed for the work, an aerated-broth-culture method and a double-agar-layer method. In the broth-culture method, phage adsorption took place in a bubbler tube containing 0.5 ml of an overnight broth culture of the recipient strain (about 2×10^9 bacteria per ml) mixed with 0.5 ml of a phage preparation from the donor strain. After 6 minutes of incubation in a water bath at 37° C, 9 ml of broth was added and the tube was aerated in the water bath until a density of approximately 2×10^9 per ml was reached, to allow for phenotypic expression of the resistant transductants. A control tube in which the bacteria were mixed with a homologous phage was treated in the same way as the transduction tube. An appropriate quantity of the antibiotic was pipetted into one side of an empty Petri dish and 0.1 ml of the culture into the other side. Then 20 ml of agar, melted and cooled to 45° C, was poured onto the drop of culture in the plate. The bacteria

and antibiotic were well mixed with the agar by movements of the plate. Three plates each were prepared from the transduction tubes and the control tubes. The plates were incubated at 37° C for 3 days, after which the colonies were counted on both series of plates.

In the double-agar-layer technique, phage adsorption was carried out in the same way as in the broth technique. After adsorption, 4 ml of broth was added before plating, in order to reduce the number of colonies developing from spontaneous mutants. Samples of 0.1 ml were taken from the tubes and pipetted onto the bottoms of empty plates. Then 20 ml of melted agar was poured into each plate and mixed well with the culture. The plates were kept at 37° C for 3 hours. They were then removed from the incubator, and 5 ml of soft agar containing the desired quantity of antibiotic was poured into each plate on top of the first agar layer. The plates were kept at room temperature for 2 hours to allow diffusion of the antibiotic, and again incubated; after 3 days the colonies were counted.

Most of these experiments were carried out with the broth technique. It was eventually realized, however, that the double-agar-layer technique is more sensitive and quantitative, and therefore preferable.

By means of the two techniques described it was possible to transduce bacteria of sensitive strains to first-step resistance to penicillin or chloramphenicol. With multistep resistant strains as donors, it was never possible to produce more than first-step resistance in transductants. In the case of penicillin resistance, it proved possible to build up multiple resistance by the transduction method. The first-step penicillin-resistant strain *pen-r-5* was transducible to second-step resistance by the first-step strains *pen-r-1* and *pen-r-10*.

The experimental data led to the following conclusions: (a) resistance to penicillin and resistance to chloramphenicol in *Salmonella typhimurium* are gene controlled; (b) polygenic systems govern such

resistance; (c) the different genes of these polygenic systems are not linked.

Transformation

For three years we have been making intermittent efforts to develop methods for applying a transformation technique in our studies of chromosomal structure in *S. typhimurium*. We had succeeded in transferring genetic markers from the wild-type strain to several mutant strains, through the medium of disintegrated bacteria of the donor type or deoxyribonucleic acid (DNA) extracted from them; but such transfers were achieved only in occasional experiments, and the frequency of success was so low that the technique could not be effectively exploited (see Year Books 54, pp. 230-231; 56, pp. 375-376). This year, however, Lahr has had good success in transformation experiments with streptomycin resistance.

A high degree of streptomycin resistance (*str-r*) was the transformation marker, and the transfer was made either by disintegrated bacteria (crude extract) or by DNA extracted from them. The recipient bacteria, in one set of experiments, carried the linked markers *leuA-39* and *ara-9*; in a second set, they were *tryD-7*; and in a third set they had the wild-type genotype. In all the experiments the donor material carried the *str-r* marker, and selection was made for streptomycin resistance transferred from donor material to recipient bacteria. The objectives were to determine (1) whether or not the *str-r* marker could be transferred and (2) whether or not the transfer was mediated by DNA.

Twenty experiments were made, and all were successful. On 42 experimental plates, 2387 *str-r* colonies were observed, whereas on 34 control plates only 21 *str-r* colonies appeared. In seven of the experiments the donor material, either crude extract or DNA, was treated with deoxyribonuclease (DNase) before being added to the bacterial suspension. These experiments produced only 14 *str-r* colonies on 16 plates;

but when the same recipient bacteria were mixed with donor material not exposed to DNase, 873 *str-r* colonies were found on 17 plates. Thus, both the DNA and the crude extract were made genetically ineffective by DNase, as is to be expected in the transformation process, in which DNA is instrumental in the transfer of genetic properties.

When the recipient bacteria in an experiment were *tryD-7*, or *leuA-39 ara-9*, the *str-r* colonies were also *tryD-7* or *leuA-39 ara-9*; therefore the *str-r* colonies could not have resulted from contamination. Efficiency of transformation was higher in the experiments with crude ex-

tract (about 3.5 per 10^6 bacteria) than in those with DNA (about 1 per 10^6 bacteria).

It was found that about 50 per cent of the *str-r* colonies also required thiamine for growth. Transduction tests showed that this requirement was due to a mutation at the *thi* locus, which is closely linked to *str-r*. Thus these experiments confirmed a previous observation, namely, that genetic markers not present in the donor bacteria may be brought into recipient cells by the transforming DNA. Such markers presumably represent mutations induced in the DNA during the extraction process.

THE NATURE OF THE MATERIALS OF HEREDITY

Berwind P. Kaufmann, Helen Gay, Kathryn Fuscaldo, and Jennie Buchanan

During the past year we have continued our studies of the patterns of organization of cellular materials. A large share of the experimental work has been devoted to analyses of fine structural detail of nuclear and cytoplasmic organelles in plant and animal cells. Electron microscopy has been used to visualize biological ultrastructure, and enzymatic hydrolysis has been applied to identify and localize chemically definable cellular constituents. The information gained provides a new frame of reference for the re-examination of problems of gene structure and function that have long engaged the attention of cytogeneticists and experimental embryologists. The combination of cytochemical and electron-microscopical methods thus serves to breach some of the barriers that have kept us in the past from acquiring a precise knowledge of the operation of genetic mechanisms in living cells of higher plants and animals.

The work of the year has been furthered by a research grant (RG-149) from the National Institutes of Health, U. S. Public Health Service. During 1957 our electron microscopy was carried out at Brookhaven National Laboratory, where Gay and Kaufmann continued to serve as Guest

Investigators. We are indebted to Dr. Howard Curtis for the privilege of using the microscope and to Dr. John Bergeron and Mr. Mark Gettner for their assistance in its operation. Since March 1, 1958, we have been privileged to work with an RCA EMU-3C microscope, obtained for our use by the Long Island Biological Association through a research grant (RG-5336) from the National Institutes of Health.

M. Gillian Pederson-Krag served as a temporary assistant during the summer of 1958. Her careful attention to operational details relieved us of many routine laboratory tasks.

Gay and Kaufmann have devoted considerable effort during recent months to the preparation of exhibits for the World's Fair at Brussels and the International Congress of Genetics at Montreal. Kaufmann has accepted appointment to the editorial boards of two new international scientific journals: *The Nucleus*, published in India, and the *International Journal of Radiation Biology*, published in England.

Patterns of Cellular Organization

Fine structure of chromosomes. On the basis of observational and experimental

evidence presented in previous Year Books, we concluded that the chromosomes in cells of higher plants and animals represent integrated fabrics of nucleic acids and proteins. The fabrics are composed, according to our cytochemical findings, of ribose and deoxyribose nucleic acids (RNA and DNA), tryptophan-containing proteins, and histones. What is more important, the amounts and patterns of association of all these materials appear to be subject to modification as the cell grows and divides, as exemplified by cyclic changes in quantity of RNA during the course of somatic mitoses, by localized accumulation and "secretion" of DNA in salivary-gland chromosomes, by increase in tryptophan-containing protein in metabolically active cells of many types, and by replacement of histone by protamine during spermatogenesis in several vertebrates. Some of these fluctuations and alterations evidently represent adaptations that facilitate the "packaging" and transmission from cell to cell of linear aggregates of essential genetic materials. But we must also seek in these cyclic alterations the role of the chromosome as a physiological unit, concerned with the control of synthetic and metabolic processes incident to the growth and differentiation of the cell and its adjustment to changing environmental conditions.

In order to seek the precise information that will enable us to define specific structural modifications in terms of the alternatives stated above, we have directed our efforts to analyzing the organization of the chromosomes during somatic and meiotic mitoses. It is generally agreed that chromosomes contain DNA, but the pattern of distribution of this nucleic acid is still subject to debate. Do the DNA molecules run the length of the chromosome, or are they attached laterally to some other backbone material (assumed to be protein)? With respect to RNA, some workers have questioned the inference that the chromosomes contain this nucleic acid.

Can we supply more convincing evidence that RNA is an essential component of chromosomes?

In extension of our studies of the distribution of DNA along the chromosome, we have repeated the radioautographic experiments begun last year, to determine the pattern of incorporation of tritium-tagged thymidine in the salivary-gland chromosomes. (These were conducted in collaboration with P. S. Woods and A. Sengün, as outlined in Year Book 56.) The DNA that is synthesized and built into the giant chromosomes in the course of larval development appears primarily in the banded regions. It is of course not possible, on the basis of this finding, to exclude the possibility that some DNA exists in the interbands. It may be present in such a low concentration that disintegrations of H^3 are rarely registered on the stripping film that serves to localize H^3 in the radioautographic techniques. When the findings are considered together with data afforded by other methods, however, it seems probable that the DNA is attached laterally to component strands, probably protein, which form the main axis of each of the constituent chromonemata. A further check of this interpretation is now in progress.

As regards the question of the presence or absence of RNA in the chromosomes, the following evidence is offered in support of our earlier contention that chromosomes contain this nucleic acid at all stages of the mitotic cycle. Our previous findings, summarized in Year Books 47 to 50, were based on hydrolysis with ribonuclease and proteases followed by staining procedures. We found that tissues fixed in acetic acid-alcohol, when digested in ribonuclease and then colored with Unna's combination stain (pyronin and methyl green), showed bright green chromosomes. In the absence of digestion by ribonuclease, the chromosomes were colored purplish. The effect of the enzyme was thus to remove a pink-staining com-

ponent from the chromosomes. The pink coloration of chromosomes by pyronin is due to the presence of RNA, and the green coloration to the presence of DNA. Our experimental evidence showed not only that RNA is found in chromosomes but also that it is intimately associated with protein (Year Book 47).

When purified crystalline deoxyribonuclease later became available for our use, we anticipated that digestion with this enzyme, in conjunction with pyronin-methyl green staining, would provide corroborative evidence of the location of RNA in chromosomes. The treatment should have produced a staining reaction in which the chromosomes were colored pink because the enzyme had removed (or degraded) the green-staining component. In our initial experiments this was indeed found to be so, but the residual pink color was very pale. With subsequent ribonuclease digestion (to show that the residual basophilic material was RNA) the pink color disappeared, but we found that some color reduction also occurred in sections immersed in the control solution (distilled water). It was therefore not possible to conclude from these experiments that the basophilia detectable after deoxyribonuclease hydrolysis is due to RNA.

In the years since these experiments were performed, many samples of pyronin from several different dye manufacturers have been subjected to various methods of purification and tested cytochemically, but none has been found wholly satisfactory for this kind of experiment. Many trials of the Azure-B technique for coloring RNA and DNA have also been made; but this metachromatic stain leaves no visible basophilia in chromosomes after digestion with deoxyribonuclease.

Recently, through the courtesy of Dr. Jean Brachet, we obtained a sample of pyronin dye produced by the J. R. Geigy Company in Basel, Switzerland. When pyronin-methyl green stain made with this

sample is applied after deoxyribonuclease treatment of cells, the chromosomes are colored brilliant pink. This colorability is almost completely eliminated by ribonuclease digestion but is not affected by control solutions. Thus we have been able to furnish convincing cytochemical evidence of the presence of RNA in chromosomes, namely, that a basophilic material, which is RNA, remains after the removal of DNA from chromosomes by enzymatic hydrolysis.

In summary, we can now demonstrate cytochemically, by two different procedures, that RNA is in chromosomes: ribonuclease treatment of untreated cells eliminates a pink-staining component from a normally purple-staining chromosome; and ribonuclease treatment of deoxyribonuclease-treated cells eliminates the residual pink colorability of the chromosomes.

Spermatogenesis in D. melanogaster. In attempting to attain the objectives outlined in the introductory paragraph of this report, we have continued the studies of spermatogenesis in *Drosophila* outlined in Year Book 56. Our observations, derived from combined electron-microscopical and cytochemical approaches, concern cytoplasmic as well as nuclear components. As the work proceeds, a more complete picture is emerging of the behavior and role of the chromosomes, nuclear membrane, mitochondria, and cytoplasmic membranes during meiotic mitoses and the consequent development of the spermatozoon. Determination of ultrastructural patterns thus promises to play an important part in reaching an understanding of the operation of genetic systems. It seems particularly important with respect to spermatogenesis in *Drosophila*, since many of our present concepts of radiation genetics rest on studies of the responses of spermatozoa of *D. melanogaster* to X rays and other types of ionizing radiations. Some aspects of our studies of spermatogenesis in *Drosophila* have been completed, and others are in progress; because of the

present gaps in our knowledge we prefer to postpone a full presentation of the findings and an appraisal of their significance.

Models from electron micrographs. Fuscaldo has worked with Henry Jones, the departmental photographer, to develop a method for preparing models of fine cellular structures from electron micrographs of serial sections. For many years anatomists have constructed models of organs and organ systems by stacking sheets of wax whose proportions and contours are determined from tracings of enlargements of serial paraffin sections. The method of Fuscaldo and Jones also involves stacked enlargements of serial sections, but photographic images are utilized rather than manual reconstructions. Because the original electron micrographs are made from extremely thin sections, it is possible by this method to determine the three-dimensional contours of intracellular structures whose small size precludes their identification by means of the ordinary light microscope.

In preparing the models, individual sections in a series are photographed with the electron microscope. The negatives are enlarged and printed on Kodak medium-contrast lantern-slide plates. The positives of consecutive serial sections are then stacked, the individual plates being affixed to one another with rubber cement. Stacks of as many as ten consecutive serial sections have been prepared in this way.

Because of the transparency of the individual plates, a stack can be illuminated from below to reveal the structural pattern resulting from superposition of the images of a number of contiguous sections. The method thus affords a more graphic representation of structural continuity than could be obtained by comparison of individual electron micrographs.

To increase the three-dimensional properties of the system, any particular stack can be photographed for viewing with the stereoscope. A holder has been designed that allows a given stack to be photographed in each of two positions, repre-

senting the interocular distance that will permit the two prints to be superimposed in stereoscopic observation.

If one wishes to examine a greater volume of a cell than can be encompassed in eight to ten serial sections (a depth of less than $0.5\ \mu$), additional stacks of eight to ten can be prepared and photographed. Eight to ten transparent positives of such photographs can then be stacked to produce a composite representing 64 to 100 serial sections and spanning a depth of about 3 to $5\ \mu$ within the cell.

The nuclear membrane as an intermediary in gene-controlled reactions. The blebbing phenomenon in the salivary-gland cells of larvae of *D. melanogaster* involves participation of the nuclear membrane in the transport of materials of nuclear origin to the cytosome. There these materials presumably participate in the synthetic processes that culminate in the production of secretion granules (Year Books 53 to 56). This system enables the genetic potentialities of a given set of chromosomes to find expression in patterns of growth and development. The reactions by which individual loci within the chromosomes express their potentialities may thus be studied in terms of the cytoplasmic activity of organelles derived from the chromosomes themselves.

We have considered the probable role of the blebbing phenomenon (and related processes) in the operation of genetic systems. Attention has been focused on the possible existence of systems for protein synthesis that involve the participation of cytoplasmic lamellae derived from detached segments of the nuclear membrane. Materials included within the detached blebs, including DNA and RNA of chromosomal origin, presumably are essential to the synthetic processes, perhaps in determining the specificity of their action. On the basis of these considerations we have suggested that the cytoplasmic membranes derived from the nuclear membrane be designated "genoplasmic membranes."

The analogy with the hypothesized plasmagene is apparent.

Perpetuation of the membranes would endow the cells with the genetic properties of the chromosomal loci responsible for their production or imprinting. Gradual loss of the membranes would lead to the disappearance of these properties (the pattern of behavior of *Dauermodifikationen*). The membranes might also serve as the progenitors of cytoplasmic particles, dependent on the nuclear genes for their maintenance and functional capacities but not for their immediate origin or specificity of action. If a given detached segment carried the imprints of several neighboring genes, it would afford a pattern for the same kind of integrated physiological system as has been suggested for the chromosome as a whole, but differing therefrom to the extent that the detached segment conveyed a portion rather than the total genetic information incorporated in the length of each of the subsidiary chromonemata. Such a system might explain, for example, the difference in phenotypic manifestation between heterozygotes having a pair of pseudoalleles located in the same chromosome and in different chromosomes. The sites of primary gene action would then be in the cytosome rather than in the chromosome as has generally been assumed.

Many other aspects of gene action are brought into better perspective when considered in terms of the coordinating functions of the nuclear membrane. In such perspective the cytoplasm assumes an essential role in integrated cellular activities. The realization that structures responsible for cytoplasmic heredity can be evoked in cells at various stages in the life of an organism serves to emphasize the harmonious interaction of nuclear and cytoplasmic processes in the operation of genetic systems. A beginning has been made in the analysis of problems of gene action by means of the electron microscope; with this approach cytogenetics finds a new

area for profitable endeavor in the study of biological ultrastructure.

Tests for Mutagenic Activity

The number of physical and chemical agents found to be effective in producing gene mutations and chromosomal rearrangements has increased greatly during recent years. Production of comparable genetic effects by many agents having diverse properties suggests in itself that mutagenic activity may proceed in the living cell by a number of different reaction pathways. What is obviously needed for further elucidation of the processes involved, as we have noted in previous Year Books, is experimentation with agents that are known to act specifically on identifiable cellular constituents. The purified crystalline enzymes meet the qualification with respect to specificity of action. We now need more precise information about the extent of their mutagenic properties.

The action of ribonuclease. It was reported in Year Books 51 to 53 that the enzyme ribonuclease (which has a molecular weight of about 13,000) can penetrate the living cell and produce chromosomal aberrations. This discovery led to an attempt to determine whether ribonuclease could induce chromosomal rearrangements in the spermatozoa of *D. melanogaster*. Among 1478 cultures tested, after treatment of males with an aerosol of ribonuclease, we found no translocations between the second and third chromosomes. It was not possible to determine from these experiments whether the aerosol failed to reach the spermatozoa or whether the enzyme was incapable of inducing chromosomal breaks. More recently we obtained evidence (Year Book 55) that ribonuclease effects an increase in meiotic crossing over in the X chromosome of *D. melanogaster*. Furthermore, a brief report of work in another laboratory suggests that the spontaneous breakage of chromosomes which occurs in some plant hybrids can be reduced appreciably by administration of excess quantities of RNA.

In the light of these observations about the essential role of RNA in the maintenance of chromosomal integrity, it seemed desirable to extend our efforts to determine whether ribonuclease has appreciable mutagenic activity. The addition of Mrs. Buchanan to our staff in March 1958 enabled us to undertake the necessary tests.

A considerable amount of time has been required to build up the stocks needed for these studies, so that only one set of data is now available. These data concern the effect of an aerosol of ribonuclease (1 mg/ml, pH 7) on the production of sex-linked recessive lethals in *D. melanogaster*. Measurements were made by the *Basc* (Muller-5) technique. Only 2 lethals were found among 1300 spermatozoa tested. One lethal was found among a comparable number of control cultures from males that had been treated with inactivated ribonuclease. It is apparent from these preliminary results that pancreatic ribonuclease is not a markedly effective mutagenic agent when administered as an aerosol. Therefore the experiments are being continued by the more laborious—but, it is hoped, more effective—method of treating the males by injecting the enzyme into the abdominal cavity.

Effect of near-infrared radiation on crossing over. Thirteen years ago we discovered that treatment of *Drosophila* males with near-infrared radiation increased by about 50 per cent the frequency of chromosomal rearrangements induced in the mature spermatozoa by a given dose of X rays (Year Book 44). No comparable effect of pretreatment with near-infrared radiation on frequency of X-ray-induced dominant or recessive lethal mutations was detected. It seemed desirable, therefore, to determine the effects of treatment of *Drosophila* females with near-infrared radiation on processes of crossing over and recombination, since breakage and reassociation of chromatids of homologous chromosomes are assumed to take place in these processes.

The experiments were undertaken in collaboration with Katherine Wilson in 1948 (Year Book 47); but the results were not reported in detail, since an over-all appraisal of the limited data then available suggested that near-infrared radiation had no significant modifying action on the processes of chromatid exchange involved in crossing over. More recent findings of other workers, and our own discovery that ribonuclease produces localized effects on recombination of chromatids in the X chromosome of *D. melanogaster* (Year Book 55), prompted us during the past year to extend and statistically evaluate our 1948 data in order to determine whether different chromosome regions vary in their response to treatment with near-infrared radiation.

The experiments were designed to measure frequency of recombination in the white, miniature, forked (*w m f*) region of the X chromosome of *D. melanogaster*. Young female pupae heterozygous for these genes were exposed to infrared radiation while kept in a moist atmosphere in vials that were immersed in a water bath maintained at 26° C. The pupae of the same age that served as controls were also kept in vials in the water bath, but were shielded from the infrared radiation. After 4 days, when all the adult females had emerged, they were mated with males carrying the recessive markers, in heavily yeasted vials in a 25° incubator. These parents were transferred at 2-day intervals, over a period of 24 days (12 transfers). The progeny from each vial was classified with respect to the different crossover and noncrossover phenotypes.

The statistical analysis was based on counts of about 6000 flies. When the infrared-treated and control populations were compared with respect to the distribution of the *w-m* and *m-f* crossover classes, a measure of heterogeneity was detected that could not be attributed to sampling errors. The over-all percentage of recombination was lower among the infrared-treated females than among the con-

trols, but this lower percentage was largely due to a reduced frequency of recombination in the *w-m* interval. In the *m-f* interval, on the contrary, the infrared-treated flies had a higher recombination value than the controls. Double-crossover recombinants were also more frequent in the infrared-treated cultures than in the controls.

The statistical significance of these differences is revealed by comparing percentages of recombination in the treated and control flies for each class of crossovers in relation to the errors of the differences. For the total crossovers, the difference divided by the standard error is 0.92; for crossovers in the *w-m* interval the quotient is 1.24; for the *m-f* interval it is 2.23; and for the double crossover, 3.06. The last two values may be regarded as significant.

The high frequency of double crossovers in the infrared-treated flies (about 8 per cent) is in itself an interesting finding. But more interesting, since it affords illuminating information about the action of infrared rays, is a comparison of the coefficients of coincidence (observed frequency of double crossovers divided by expected frequency) for the treated and control groups of flies. For the control group this coefficient is 0.724 (a value almost identical with that obtained in our previous studies of the effects of ribonuclease on recombination in the *w m f* region). For the infrared-treated group the index is 0.944. This means that inter-

ference was largely eliminated. A crossover in the *w-m* region did not interfere with the chances of a crossover in the *m-f* region (or vice versa) to the same degree in the infrared-treated females as in the untreated controls.

The results thus suggest that, although near-infrared radiation does not promote "breakage" of chromatids, it does so alter the physical and chemical properties of the chromosome as a whole that the recombinational events in one region do not appreciably alter the recombinational potentialities in an adjoining region. This modification of properties might be visualized in terms of mechanical models, as has been done so frequently in the past; but the fact that each chromatid involved in crossing over represents a compact bundle of cytologically separable strands (Year Books 55, 56) suggests that attention should preferably be focused on the patterns of association and bonding of the constituent nucleoproteins. Infrared radiation promises to be extremely useful in such an analysis. Assuming that specific wavelengths can be shown to produce designated biological effects, it should be possible to obtain useful information about molecular structure by correlating biological assay with infrared spectroscopy. For the moment, however, we need to substantiate the findings reported above by a more extensive study of the effects of near-infrared radiation on the phenomena of crossing over and recombination.

INTRACELLULAR DEOXYRIBONUCLEASES

Margaret R. McDonald and Judith Karossa

Enzymes capable of depolymerizing deoxyribonucleic acid (DNA) occur in a wide variety of cells, both in microorganisms and in tissues of higher plants and animals, and are probably ubiquitous cell constituents. Only the depolymerase secreted by the pancreas (whose function is primarily digestive) has been crystallized. The intracellular deoxyribonucleases (DNases), which appear to be essential in the

synthesis of materials of genetic significance, represent a functionally and chemically distinct group of enzymes, endowed with a high degree of specificity. Duplication of DNA-containing structures may be assumed to be one of the necessary steps preceding cell division. The mechanisms by which the cell synthesizes these macromolecules are still unknown, but the intracellular DNases are probably in-

volved. Until they are isolated and characterized, their precise role in life processes cannot be established. We have therefore concentrated our efforts this year on ascertaining the best procedures for extraction and purification of one of this group of enzymes.

As was mentioned in Year Book 56, we have found salmon testes to be an excellent source of intracellular DNase. We have determined that, on a wet-weight basis, it is three to five times more potent than calf spleen, which, in turn, is two to three times more potent than calf thymus; these two organs have heretofore been regarded as the best sources of intracellular DNases. Furthermore, we have been able to find very simple conditions for extraction of the enzyme from salmon testes. These procedures yield over 90 per cent of the total activity present, and less than 10 per cent of the water-soluble proteins—an immediate tenfold enrichment by comparison with aqueous extracts of the tissue. Methods that have been developed for further purification achieve an additional sixtyfold enrichment without any appreciable loss of enzymatic activity.

The final product contains at least three proteins, as determined by paper electrophoresis. One of these has been crystallized, but preliminary assays indicate that it is not DNase. A "heme" pigment, which may be an essential prosthetic group of salmon testes DNase, is concentrated simultaneously with the enzyme.

The extraction and purification procedures, as developed so far, are described below. Other methods are being elaborated for still further purification of the enzyme, in preparation for a detailed study of its properties, mode of action, and precise relation to cell division and the metabolism of DNA.

Purification of Deoxyribonuclease from Salmon Testes

Unless otherwise specified, all operations are performed at 1° to 4° C and all filtrations are done with suction. The concentration, yield, and degree of purification attained at each step of the DNase preparation are summarized in table 7.

Step 1. Extraction and concentration. Fifty pounds of frozen salmon testes is thawed for 24 hours; freed of blood vessels,

TABLE 7. Summary of Procedure for Extracting and Purifying Deoxyribonuclease from Salmon Testes

Fraction	Total Activity,* 10 ⁴ units	Total Protein,* mg	Specific Activity, units/mg protein	Yield, %
1. Preliminary purification and concentration:				
Acid extract	857 †‡	21,900 †	391	
0.80 sat. (NH ₄) ₂ SO ₄	967 ‡	2,580	3,760	
0.65 sat. (NH ₄) ₂ SO ₄	1100	1,950	5,640	100
2. Fractional precipitation:				
Aqueous extract of 0.65 sat. (NH ₄) ₂ SO ₄ precipitate	1110	1,240	8,950	101
0.00–0.70 sat. (NH ₄) ₂ SO ₄	1050	993	10,600	95
0.45–0.70 sat. (NH ₄) ₂ SO ₄	993	407	24,400	90

* Based on 50 pounds of salmon testes.

† Over 90 per cent of the activity present in the minced (or homogenized) testes is found in the acid extract, with less than 10 per cent of the water-soluble proteins.

‡ The value for the total activity of the 0.65 saturated (NH₄)₂SO₄ precipitate is consistently higher than that of the 0.8 saturated (NH₄)₂SO₄ precipitate, which in turn has higher activity than the acid extract. A possible explanation may be that an inhibitor, present in the initial extract, is lost upon fractionation.

connective tissue, and other extraneous matter; and minced in a meat grinder. The mince is suspended in 50 liters of 0.05 *N* H₂SO₄ and left, with occasional stirring, for 6 to 8 hours. The suspension is then filtered overnight by gravity through fluted papers (Whatman no. 12, 50 cm).

The filtrate (about 54 liters) is brought to 0.8 saturation of (NH₄)₂SO₄ by the addition of 561 grams of salt per liter. A slight precipitate forms. This is collected by filtration through soft paper (Eaton-Dikeman no. 612) with the aid of 0.5 gram of Standard Super-Cel per liter of suspension. The filtrate is discarded. The filter cake is suspended in 1000 ml of H₂O and refiltered through soft paper. This process is repeated twice, with 300 ml and 200 ml of H₂O. The residue is discarded; the extracts are combined and brought to 0.65 saturation of (NH₄)₂SO₄ by the addition of 430 grams of salt per liter. The resulting suspension is filtered through hardened paper (Schleicher and Schuell no. 576); the filtrate is discarded; and the filter cake (about 13 grams) is stored at 1° to 4° C until material from at least 150 pounds of testes has been collected.

Step 2. Fractional precipitation with ammonium sulfate at 20° C. The combined 0.65 saturated (NH₄)₂SO₄ filter cakes from step 1 are suspended in 5 times their weight of H₂O and filtered through soft paper with the aid of 1 gram of Standard Super-Cel per 100 ml of suspension. The residue is discarded. The filtrate is brought to 0.7 saturation by the addition of 233 ml of a saturated solution of (NH₄)₂SO₄ per 100 ml of filtrate. The precipitate is collected by filtration through hardened paper, and the filtrate is discarded. The filter cake (approximately 6 grams per 50 pounds of salmon testes) is dissolved in 5 times its weight of H₂O, and the solution is brought to 0.45 saturation by the addition of 82 ml of saturated (NH₄)₂SO₄ per 100 ml of H₂O. The suspension is filtered through hardened

paper and the residue discarded. The filtrate is then brought to 0.7 saturation by further addition of 83 ml of saturated (NH₄)₂SO₄ per 100 ml of filtrate. The precipitate is collected by filtration through hardened paper. Approximately 2 grams of filter cake is obtained from each 50 pounds of salmon testes.

"Inert" Crystalline Protein

The final DNase preparation from step 2 contains at least three proteins, as determined by paper electrophoresis. One of these has been crystallized by the procedure described below. Although the initial crystals show DNase activity, it is undoubtedly due to contamination by the mother liquor, for their specific activity is lower than that of the latter and decreases upon recrystallization (table 8). Although

TABLE 8. Deoxyribonuclease Activity of "Inert" Protein Crystals

Fraction	Total Activity,* 10 ³ units	Total Protein,* mg	Specific Activity, units/mg protein
0.45-0.7 sat. (NH ₄) ₂ SO ₄	9930	407	24,400
First crystals	3080	220	14,000
First mother liquor	6260	183	34,200
Second crystals	283	93	3,040
Second mother liquor	2490	124	20,000

* Based on 50 pounds of salmon testes.

removal of this crystalline protein improves the quality of the DNase preparation, it has not yet been incorporated into the general purification procedure, since the loss of DNase activity (although recoverable) and the time and effort involved do not appear at the present time to be justified by the small (1.4 fold) gain in enrichment.

Crystallization of "inert" protein. The 0.7 saturated (NH₄)₂SO₄ filter cake from step 2 is dissolved in 1.5 times its weight of H₂O. The solution is adjusted to pH

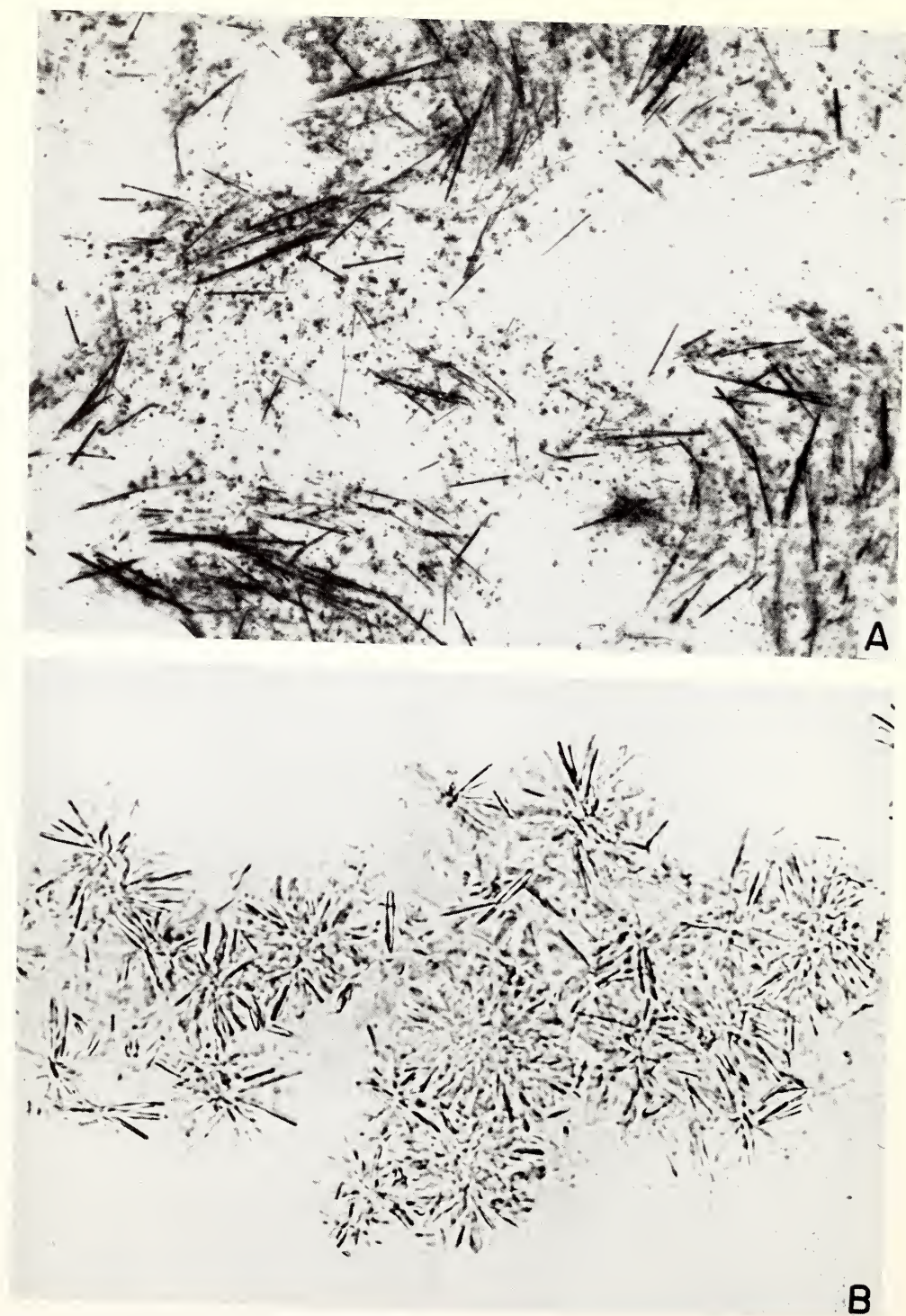


Plate 1. Crystals of "inert" protein from salmon testes: *A*, once crystallized; *B*, thrice crystallized.

3.8 by the dropwise addition of 1 *N* H₂SO₄, and clarified, if necessary, by centrifugation. Saturated (NH₄)₂SO₄ is then added, to the point of incipient turbidity, and the mixture is left at 20° C. A thick suspension of fine needles and amorphous material gradually forms (pl. 1, *A*). After 1 week the mixture is centrifuged. From 60 to 70 per cent of the DNase activity remains in the supernatant (table 8). The

residue is suspended in a volume of H₂O just sufficient to dissolve the crystals, leaving most of the amorphous material undissolved, and the suspension is recentrifuged. Saturated (NH₄)₂SO₄ is again added, to incipient turbidity, and the mixture is left at 20° C. Clusters of fine needles rapidly form (pl. 1, *B*). Crystallization appears to be complete in 48 to 72 hours.

THE SUPPRESSOR-MUTATOR SYSTEM OF CONTROL OF GENE ACTION IN MAIZE

Barbara McClintock

Continued attention has been given during the past year to the manner in which controlling elements in maize affect the phenotypic expression of known genes. Controlling elements, because of their capacity for transposition, are considered as chromosome components apart from the genes, which appear to be stationary. Once this distinction had been made it was possible to examine the mode of operation of controlling elements independently of genes and to study relations among them. It was found that some elements interact with others to control gene expression. In this respect, the elements can be segregated into groups whose members interact with one another but not with members of other groups. Each group exhibits a particular type of control of gene expression. Therefore, a group of interacting elements has been referred to as an operational system. Progress has been made in interpreting the mode of operation of several of these systems, but some important questions about controlling elements themselves remain unanswered. We do not know, for example, how they are incorporated in a chromosome or by what process transposition is accomplished. Experiments aimed at answering such important questions are needed. My attention has not been turned in these directions, because I believe that we should first expand our knowledge of different systems of controlling elements in order to

learn, if possible, whether the modes of operation of all controlling elements are basically alike. It is conceivable that these elements may be composed of one common type of substance and have one common mode of operation, as is now thought to be true of genes.

Appreciation of the way in which some systems operate may be gained rapidly; with other systems, analysis may be difficult and progress slow. A system may be responsible for the appearance of an array of quite different phenotypes, even within a single plant. Also, in some cases, analyses may be complicated by what appears to be an irregular form of inheritance of components of the system. That apparent complexities in phenotypic expression can be resolved into ordered patterns when the operation of a component of a system is comprehended became apparent during the course of studies conducted this year; the findings will be considered in the discussion that follows.

The Mode of Operation of the Spm Element

The relation between a_1^{m-1} , located in chromosome 3, and a_2^{m-1} , located in chromosome 5, was considered in Year Book 56. It was mentioned there that the action of genic substance at each of these two loci is under the control of elements belonging to the *Spm* (Suppressor-mutator) system, and, further, that the *Spm* element in a_2^{m-1}

cultures does not behave quite like that in a_1^{m-1} cultures. During the past year, differences between these two elements have been examined. As a consequence, some previously puzzling aspects of the system of control of gene expression in a_2^{m-1} cultures have been clarified.

The difficulties encountered in earlier attempts to analyze the system of control of gene expression at a_2^{m-1} were not experienced in the analysis of a_1^{m-1} . Whereas in the a_2^{m-1} cultures detection of the *Spm* element was much delayed, *Spm* was easily identified in the a_1^{m-1} cultures. Its mode of operation in these cultures could be readily discerned, and its inheritance behavior did not seem complicated. Transposition of the element from one location to another in the chromosome complement could also be followed easily. It was learned, however, that this *Spm* element is not altogether stable. Some changes in its action were recognized; and one of these, effecting a reduction in its capacity to inhibit gene expression at a_1^{m-1} and to cause mutation at that locus, was discussed in Year Book 56. This finding suggested the possibility of a direct relation between the degree of suppressive capacity of an *Spm* element and its ability to induce mutation.

Most of the studies of control of gene expression at a_1^{m-1} were conducted not with the modified *Spm* element, just mentioned, but with the originally identified *Spm* element, whose capacity to suppress the expression of gene action at a_1^{m-1} is complete. In the presence of this *Spm* element there is no visible evidence of action of the genic substance of the a_1^{m-1} locus except in those areas of a plant or kernel that arise from cells in which mutation to or toward A_1 has occurred. The cells having such mutations contain anthocyanin pigment; and the mutants so formed are thereafter stable. The phenotype attributable to a particular mutation will be produced by the mutant either in the presence or in the absence of *Spm* in subsequent generations of plants.

It was found that another type of change also can occur at the locus of a_1^{m-1} . Each such change is reflected in an altered response of a_1^{m-1} to given conditions in subsequent cell and plant generations. The alteration must modify some component of the locus that is capable of being reproduced during each chromosome replication; in other words, the modification effects a heritable change in the state of the locus. Control of the time of occurrence of mutation during development of a tissue, of the type of mutation, and of the number of cells in which mutation takes place, was found to reside at the locus of a_1^{m-1} , each state expressing a particular mutational pattern in the presence of *Spm*. The pattern peculiar to each state is not altered by increased doses of *Spm*. The same pattern appears when one or more than one *Spm* element is present. In the absence of *Spm*, on the other hand, some gene action at a_1^{m-1} is expressed as anthocyanin pigmentation in both plant and kernel by all but one of the examined states, and the degree of this action, as indicated by the intensity of pigmentation, is also a reflection of the state of a_1^{m-1} . In the absence of *Spm*, however, no mutations occur and each state retains its integrity through successive plant generations. In short, the expression of types of mutation and of their pattern of distribution in plant or kernel in the presence of *Spm*, and also the type of gene action exhibited in its absence, define a state of a_1^{m-1} . No unresolved ambiguities in this respect were encountered during an extensive study of states of a_1^{m-1} .

In the a_2^{m-1} cultures, in contrast, such clear-cut distinctions between states were not evident in the early studies. It was realized, nevertheless, that not all derivatives of the original a_2^{m-1} were alike. At first, the different isolates could be segregated only into two main classes. Difficulties were encountered in interpreting the mode of control of gene expression of members within each class. A few examples will be given of the irregular pat-

terns of gene expression observed in members of the first class.

In plants carrying isolates of a_2^{m-1} belonging to the first class, it was noted that abruptly initiated changes of some kind, apparently occurring within individual cells, resulted in the appearance of well defined areas in which the phenotypic expression of gene action at a_2^{m-1} differed from that in the surrounding tissue. Such areas also appeared in ears, all the kernels within an ear exhibiting the modified type of gene expression. Again, such modified areas were often seen even within a single kernel. Different types of modification were sometimes observed in the same plant. Anthocyanin pigment would be totally absent in some areas whereas most of the rest of the plant was deeply pigmented; or the intensity of pigment within an area might simply be reduced. Areas were also noted in which streaks of deep pigmentation appeared in an otherwise nonpigmented background. Both the number of these deeply pigmented streaks and their size seemed to be controlled in some manner, for each such area exhibited a precise pattern in these regards. The patterns differed among the areas, however, and often over a very wide range. Some areas had only a few small pigmented streaks; others, many such small streaks. In still others, the size of the streaks was much larger, and there might be either a few or many within one area. In the course of study of isolates belonging to the first class, it was found that mutation to or toward A_2 occurred, but only in those cells of an area that otherwise exhibited no anthocyanin pigment. It was also found that the mutants were thereafter stable with respect to the phenotypes they produced. No variegated phenotypes appeared in either a plant or a kernel having one of the mutations, when tested under conditions that would be expected to reveal any instability of expression had it occurred.

Often, in plants showing the above-described irregular patterns of distribution of anthocyanin pigment, similar irregulari-

ties were noted among the kernels. Some kernels were uniformly pigmented, and in most of them the intensity of pigmentation was low. Others were variegated, exhibiting deeply pigmented spots in a colorless background. Sometimes all the variegated kernels on an ear exhibited similar patterns with respect to size and number of such spots, but on other ears wide differences were observed among the variegated kernels. Some had only a few small specks of deep pigmentation, others had many such specks; in some, a number of medium-sized pigmented spots appeared, and in still others there were relatively few spots but most of them were large. Very often, on an ear produced from a testcross, the ratio of variegated to pale-colored kernels, and also the proportions of different kinds of variegated kernels, gave no evidence of orderly meiotic segregation of heritable elements that might be responsible for the appearance of the different kinds of kernels. On some other ears, in contrast, the ratio of kernel types was quite consistent with an orderly segregation. Frequently, the different ears produced by one plant were not at all consistent in this regard, even when the same pollen parent had been used in making the cross to each of the ears.

Confused impressions of the operation of the system of control of gene expression at a_2^{m-1} also resulted from attempts to analyze the constitution of plants derived from the pale-colored kernels on ears that segregated both pale-colored and variegated kernels. Some of these plants had variegated phenotypes. Others, however, showed no evidence of variegation, the anthocyanin pigment being uniform in intensity and distribution throughout the plant. Study of the progeny of these plants led to further difficulties of interpretation. When the ears of one such plant were used in crosses with tester plants homozygous for the stable recessive, a_2 , all the pigment-containing kernels on each ear of one plant might be uniformly pale colored; whereas, in another plant, one ear

might have all uniformly colored kernels and another ear might have also a few variegated kernels. These variegated kernels exhibited deeply pigmented spots in a colorless background; the pattern of spots might be the same among all, or might differ widely. On still another ear of the same plant, a clear-cut Mendelian ratio of pale to variegated kernels might appear.

That some system of control of gene expression was operating in the case of a_2^{m-1} was clear, but evidence from the early studies did not allow ready recognition of its components. The above-described examples of irregular phenotypic expressions and inheritance patterns observed in plants carrying a class I state of a_2^{m-1} may suggest why this was so. Only when the behavior of the state of a_2^{m-1} belonging to the second class was being examined was initial evidence obtained of the presence in this system of a heritable element responsible for suppressing phenotypic expression of a_2^{m-1} . In this aspect of its operation, it resembled the *Spm* element previously discovered in the a_1^{m-1} cultures. We now know that this resemblance is more than coincidental. These elements are basically alike, and it is altogether probable that they arose from a common progenitor. Each appears to represent a different state of one controlling element. The following discussions will consider the differences between them, and will explain the confusion experienced in earlier attempts to interpret the operation of the system responsible for controlling gene expression at a_2^{m-1} .

After it was recognized that an *Spm*-type element was involved in this system, the *Spm* element extensively examined in the a_1^{m-1} cultures was introduced into some plants having one of the states of a_2^{m-1} belonging to class I, and subsequently into plants carrying other states of this class. With this *Spm* element present, the mode of control of gene expression was as sharply revealed as it had previously been in the case of a_1^{m-1} . Moreover, some states of a_2^{m-1} were seen to resemble some states

of a_1^{m-1} . In the absence of *Spm*, both plants and kernels having one of these class I states of a_2^{m-1} were pigmented. The intensity of anthocyanin pigmentation in plants was rather high, whereas in kernels it was always low; and the distribution of pigment was uniform in both plant and kernel. In the presence of *Spm*, no pigment appeared except in those areas of plant or kernel that arose from cells in which mutation to or toward A_2 had occurred. Such mutations occurred in some germinal cells, and, as a consequence, mutants could be isolated. They were found to be stable in expression in subsequent generations of plants, either in the presence or in the absence of *Spm*.

Through the use of this *Spm* element, it was learned that class I states of a_2^{m-1} differ from one another in the manner in which they control the time and frequency of occurrence of mutation during development of plant or kernel; and some sharply expressed distinctions among them were noted. These class I states of a_2^{m-1} and the states of a_1^{m-1} are much alike. The similarities include the appearance of uniformly distributed anthocyanin pigment in plant and kernel, and stability of this phenotypic expression in successive generations of plants, in the absence of *Spm*; suppression of all visible evidence of this kind of gene action in the presence of *Spm*; and also control of mutation type and pattern by the state when *Spm* is present. The *Spm* element originally present in the a_2^{m-1} cultures, on the other hand, did not always effect suppression of gene action in all parts of a plant or kernel. Mutations, however, occurred only in those parts of plant or kernel in which gene expression was suppressed. The pattern of mutation produced by any one state was not set, and the types observed ranged from early-occurring mutations, which resulted in the appearance of large areas of mutant phenotype, to late-occurring mutations, which produced only specks of deep pigmentation. Invariably the mutations were confined to regions in which the background

phenotype was nonpigmented, that is, in which the suppressive component of *Spm* activity was evident.

It is now apparent that one of the major difficulties in analyzing the control system responsible for the many different patterns of a_2^{m-1} gene expression was occasioned by alternating phases of activity of the *Spm* element in the a_2^{m-1} cultures. But one other factor also contributed to the confusion. Early in the study of a_2^{m-1} , a new type of state appeared, whose expression was in great contrast to those observed with other isolates of a_2^{m-1} . It was therefore set apart and designated as the class II state of a_2^{m-1} . By means of various kinds of experiment with this state, it was first learned that the *Spm* element in the a_2^{m-1} cultures may undergo frequent changes in activity during the development of a plant, each such change affecting its capacity to serve as a suppressor-mutator. Clearly, some regulatory mechanism controls the time of occurrence of such changes, although it is not yet understood. Such a change may evidently occur within an individual cell during development, for well defined sectors in a plant or kernel often exhibit the result. The direction of change may be from active to inactive, or from inactive to active, and alternating cycles may also be observed. The class II state of a_2^{m-1} readily reveals these changes in action capacity of *Spm*, for, with this state, the *Spm* element in its active phase serves only to inhibit expression of gene action at a_2^{m-1} . No evidence of mutation has yet been obtained with this state, although abundant evidence has been obtained with the class I states. It is important to bear this fact in mind in order to appreciate the significance of the phenotypes arising from the class II state, which will be considered below. All the variegated patterns to be described reflect only changes undergone by the *Spm* element itself.

The class II state of a_2^{m-1} . When *Spm* is absent, or when an *Spm* element is present in its inactive phase, the class II state

of a_2^{m-1} produces a phenotype similar to that given by standard A_2 . Both kernel and plant are deeply pigmented. In a plant that is a_2^{m-1} (class II)/ a_2 in constitution and carries a single active *Spm* element derived from the a_2^{m-1} cultures, suppression of the effects of a_2^{m-1} gene action is not complete. Pigment is present, but it is less intense than the pigment of plants having no *Spm* or *Spm* in its inactive phase. A change in *Spm* from an active to an inactive phase during the development of such a plant is made evident by a well defined area of very deep pigmentation in a more lightly pigmented background. The positions of such areas in a plant, their number, and their relative sizes reveal both time and frequency of occurrence of such changes in *Spm* activity during development. If the *Spm* element is inactive during early development, a change from the inactive to the active phase is represented by areas of lower pigment intensity in the background of intense pigmentation. In kernels, on the other hand, this *Spm* element in its active phase will suppress all expression of gene action at a_2^{m-1} . Those parts of the kernel in which it is active are totally colorless, but other parts, where it is inactive, are intensely pigmented. Changes in *Spm* action phase may alternate, and both the times and the types of change are revealed in the kernel phenotypes. In kernels having one *Spm* element, these alternating changes may be observed readily. For example, a large pigmented area may be seen in an otherwise colorless region of a kernel. Within this large pigmented area, smaller colorless areas may be observed, and within these, in turn, specks of deep pigmentation. In this illustration, the sequence of changes of phase of *Spm* activity during development of the kernel was from active to inactive to active, and again to inactive.

The dose of *Spm* can alter the patterns of variegation produced by the class II state, and this dose effect can be seen in

both plant and kernel. It is particularly well expressed in the aleurone layer of the endosperm of the kernel. The endosperm is triploid and therefore is useful for examining the effects produced by various doses of any chromosomal component. The female gametophyte contributes two genetically identical haploid nuclei to the primary endosperm nucleus, and the pollen grain contributes one haploid nucleus. The different patterns of variegation exhibited by kernels having one, two, or three active *Spm* elements provide a means of determining whether a plant has no *Spm* element or an *Spm* element in its inactive phase. Before explaining this method of differentiation, it is necessary to review some of the tests that have been made with the class II state of a_2^{m-1} .

The first example concerns a plant that carries this state and also carries one *Spm* element, which was in its active phase in the cells that produced a particular ear of the plant. The endosperm of any kernel appearing on that ear after pollination should have received from the female parent either no *Spm* or two active *Spm* elements; and the ratio of this distribution of *Spm* among the kernels is expected to be 1 to 1. If the plant serving as pollen parent for this ear is homozygous for a_2 and has no *Spm* element, half the a_2^{m-1} -carrying kernels produced by the cross should have no *Spm*, and half should have two *Spm* elements. Kernels of the former type should be deeply and uniformly pigmented, and those of the latter type should reveal the presence of the active *Spm* elements. In the many tests of this kind so far conducted, the *Spm*-carrying kernels have shown a number of rather small, deeply pigmented spots in a colorless background, and the ratio of fully pigmented kernels to variegated kernels has been 1 to 1. When the reciprocal cross is made, a 1-to-1 ratio of deeply pigmented to variegated kernels again results. In this case, however, the variegated kernels show a number of large, deeply pigmented areas, within which smaller, colorless areas often

appear. It has been learned from other tests, one of which will be described below, that this is the characteristic pattern of variegation in kernels that start development with one active *Spm* element in the primary endosperm nucleus.

In a cross similar to that described above except that *Spm* is absent in the ear-producing parent, all the a_2^{m-1} -carrying kernels on the resulting ears should be deeply and uniformly pigmented, for none of them will have an *Spm* element. If, however, the pollen parent that is homozygous for a_2 has one active *Spm* element, half its pollen grains should have no *Spm* and half should have one *Spm*. Two types of a_2^{m-1} -carrying kernels are expected to appear on an ear produced by this cross, and they should be present in equal numbers. Those with no *Spm* should be deeply and uniformly pigmented, and those with one *Spm* should be variegated. More than fifty crosses of this kind were made, and all the ears so produced had a 1-to-1 ratio of uniformly pigmented to variegated kernels. In all cases, the variegated kernels exhibited pigmented spots in a colorless background; many of these spots were large. Within most of the large pigmented areas, smaller colorless spots were present, and some of these contained specks of pigment.

If pollen from a plant homozygous for a_2 and carrying one active *Spm* element is placed on the silks of an ear of a plant homozygous for the class II state of a_2^{m-1} and carrying one *Spm* element that was in its active phase in the cells that gave rise to the ear, then four types of kernels appear in equal proportions. One type is fully pigmented (no *Spm*); another shows many pigmented areas, many of them large, in a colorless background (one *Spm*); a third has pigmented spots, all rather small, in a colorless background (two *Spm*); and a fourth shows only small specks of pigment in a colorless background (three *Spm*). Higher doses of *Spm* elements in the endosperm have been produced by other kinds of testcrosses.

These tests have shown that kernels having four *Spm* elements exhibit at the most one or a few tiny specks of deep pigmentation; much more often they are totally colorless. Kernels having more than four *Spm* elements are all totally colorless.

The relation between pattern of variegation and dose of *Spm* was confirmed by tests in which the location of *Spm* in the chromosome complement was known because of its linkage with some genetic marker. The distribution of the linked marker among the various categories of variegated kernels was that expected in the event of a direct relation between pattern of variegation and dose of *Spm*; an illustration will be given later. Confirmation was also obtained in tests of the *Spm* constitution of plants derived from the variegated kernels of each category.

As was mentioned earlier, the relation between pattern of variegation and dose of *Spm* has made it possible to determine whether a plant whose phenotype exhibits no evidence of *Spm* actually has no *Spm* or carries an *Spm* element in an inactive phase. This procedure will now be described.

Among a_2^{m-1} -carrying plants, those having an inactive *Spm* element and those having no *Spm* element may be phenotypically the same. *Spm* may be present although no evidence of it appears in any part of the plant. In other plants, there is no sign of the presence of *Spm* in the main stalk but one or more of the tillers have sectors of the phenotype produced by an active *Spm* element. Several kinds of test may be employed to determine whether or not *Spm* is present in a plant, or parts of a plant, where it is not revealed phenotypically. The most significant of these was formulated after it was discovered that the introduction of an active *Spm* element into a nucleus carrying inactive *Spm* elements will result in activation of the latter. In this test, ears for crosses are selected in plants showing no evidence at all of *Spm*, or in parts of a plant that

show no evidence of *Spm* although it is known to be present in other parts of the plant. When possible, the first and second ears produced by the main stalk are used.

The silks of one ear of a plant receive pollen from a plant that is homozygous for a_2 , and for other recessive markers if these are useful for the test, but that carries no *Spm*. If *Spm* is absent in the female parent, or if it is present but was in its inactive phase in the cells that gave rise to the ear used in the cross, all the a_2^{m-1} -carrying kernels on this ear will be deeply and uniformly pigmented. Silks of a second ear of the same plant receive pollen from a plant of the same constitution as the first pollen parent except that it carries one active *Spm* element. Half its pollen grains will carry an active *Spm* element, and half will have none. If *Spm* is absent in the female parent, two types of a_2^{m-1} -carrying kernels will appear on the second test ear, in equal frequencies. One type will have deep pigmentation, uniformly distributed over the aleurone layer (no *Spm*). The other type will be variegated, with a colorless background and many pigmented areas in a pattern that characteristically appears when only one active *Spm* is present. If the ear-producing parent carries an inactive *Spm* element, however, three instead of two types of kernel will appear on the ear produced by this second cross. Half the kernels will be deeply and uniformly pigmented; the other half will be variegated, and will fall into two sharply defined subclasses with equal numbers of kernels in each. One subclass will show the heavily variegated pattern characteristically produced when one active *Spm* element is present, and the other will show the pattern produced when three active *Spm* elements are present. All the variegated kernels receive one active *Spm* element from the pollen parent. The differences in pattern among them discriminate between those that receive no *Spm* from the female parent (the one-*Spm* pattern) and those that receive two inactive

Spm elements from the female parent (the three-*Spm* pattern). The three-*Spm* pattern appears because the two inactive *Spm* elements derived from the female parent are activated by the *Spm* derived from the male parent. Among the fully pigmented kernels, half receive no *Spm* from the female parent and half receive the two inactive *Spm* elements. In the latter category, the *Spm* elements remain inactive during the development of the kernel, for no active *Spm* element is introduced into the primary endosperm nucleus by the male parent to trigger them into activity.

Evidence to confirm the above analyses of *Spm* constitution among kernels of different types was derived from tests of a_2^{m-1} -carrying plants in which one inactive *Spm* element was present and was located near *wx* in one of the two chromosomes 9; the homologous chromosome 9 carried *Wx*. The results of tests conducted with one plant may be examined for illustrative purposes. Two ears of a plant that was a_2^{m-1} (class II) *Bt/a₂ bt, Wx + /wx Spm* (inactive) were crossed by a plant homozygous for a_2 , *bt*, and *wx* and carrying no *Spm* element. (The locus of *Bt*, normal endosperm, and its recessive allele, *bt*, brittle endosperm, is close to the centromere in the long arm of chromosome 5, and it undergoes approximately 6 per cent crossing over with the locus of a_2^{m-1} in the short arm of that chromosome.) None of the a_2^{m-1} -carrying kernels on these two ears was variegated. All the kernels were deeply and uniformly pigmented, and the ratio of *Wx* to *wx* among them was 1 to 1.

A third ear of this plant was crossed by a plant that, again, was homozygous for a_2 , *bt*, and *wx*, but had one active *Spm* element. (Twenty-eight testcrosses had been made to determine the presence and activity of the *Spm* element in this pollen parent. Among 5997 kernels produced in these tests, 3041 had received no *Spm* element from the pollen parent and 2956 had received an active *Spm* element. It could therefore be assumed that half the pollen

grains of this plant had no *Spm* and half carried an active *Spm* element.) When pollen of this plant was used on the third ear of the plant under consideration, 468 kernels were produced. Among them, 245 were completely colorless—34 *Bt* (18 *Wx* : 16 *wx*) and 211 *bt* (105 *Wx* : 106 *wx*). Undoubtedly, these were homozygous for the stable recessive, a_2 . The remaining 223 kernels (195 *Bt* : 28 *bt*) had pigment and so had received a_2^{m-1} from the female parent. Among them, 114 were deeply and uniformly pigmented (57 *Wx* : 57 *wx*); the other 109 were all variegated, with pigmented areas in a colorless background. In 57 of these, the pattern of variegation was characteristic of the presence of one *Spm*; 45 of them were *Wx* and 12 were *wx*. The other 52 variegated kernels had only specks of pigment in a colorless background, the characteristic pattern produced when three *Spm* elements are present; only 9 of these were *Wx*, whereas 43 were *wx*.

The ratio of kernel types on this third ear, and the relation between pattern type and the alleles of *Wx* among the variegated kernels, indicated that the female parent had some component in the *wx*-carrying chromosome, and closely linked with *wx*, that was responsible for change of pattern from a one-*Spm* type to a three-*Spm* type. That this was the *Spm* element in its inactive phase was indicated by two kinds of test. One was conducted with sib plants and with related plants in which an *Spm* element was obviously present and active. These plants were either a_2^{m-1} *Bt/a₂^{m-1} Bt* or a_2^{m-1} *Bt/a₂ bt*, and all were *Wx/wx*. The silks of ears of these plants received pollen from plants homozygous for a_2 , *bt*, and *wx* but having no *Spm*; and this set of testcrosses produced a total of 3007 a_2^{m-1} -carrying kernels. In 1538 of them, the aleurone layer was deeply and uniformly pigmented; 1345 of these were *Wx* and 193 were *wx*. The remaining 1469 kernels were variegated, with pigmented areas in a colorless background; 166 were *Wx* and 1303 were *wx*. Relatively close linkage of *Spm*

with *wx* was demonstrated by the ratios of kernels on each of the ears.

The second kind of test confirming the presence of an *Spm* element in its inactive phase was much more direct. It was made with sib plants of the one considered above. In them, the phenotype of the main stalk showed no signs of *Spm*, but one of the tillers gave obvious evidence of its presence, in an active phase. The two kinds of cross described above were conducted with ears of the main stalk on such plants. The results in each case were similar to those described: no evidence of *Spm* when the pollen parent contributed no *Spm*, and evidence of an inactive *Spm*, closely linked to *wx*, when the pollen parent contributed *Spm*. The presence in the main stalk of an inactive *Spm* element, closely linked with *wx*, was inferred. Confirmation came from tests of the constitution of tiller ears of such plants. They were used in crosses with a plant homozygous for *a₂*, *bt*, and *wx*, in which no *Spm* was present. Segregation of kernel types on these ears clearly indicated that the *Spm* element in the tiller that produced each of them was located close to *wx* in one chromosome 9. There appears to be little doubt, then, of the effectiveness of the described tests in revealing whether *Spm* is absent in a plant or is present in its inactive phase. The tests have also shown that a change in phase of *Spm* is not associated with a detectable change in its location in the chromosome complement. Evidence of transposition of the *Spm* element has been obtained; but the observed changes in location have not altered the expression of its cyclically occurring changes in action phase.

Tests of Spm activity in plants having class I states of a_2^{m-1} . The deeply pigmented areas in a colorless background observed in kernels having the class II state of a_2^{m-1} and an active *Spm* element are an expression of events that affect only the *Spm* element. They do not express mutations to stable *A₂*, as do the deeply pigmented areas that appear in kernels having a class

I state of a_2^{m-1} . Therefore, the class II state has been particularly useful in elucidating changes in phase of activity of the *Spm* element, as described in the previous section. Nevertheless, similar analyses may be conducted with the class I states of a_2^{m-1} if attention is focused on the patterns of palely pigmented areas that may also be present in kernels exhibiting the deeply pigmented spots that represent mutation-producing events at a_2^{m-1} .

As was stated earlier, kernels carrying class I states of a_2^{m-1} have uniformly pale pigmentation if *Spm* is absent. The same phenotype will appear if *Spm* is present but in an inactive phase. Thus, the pale-pigmented phenotype in the kernels carrying the class I states of a_2^{m-1} is the counterpart of the deeply pigmented phenotype in kernels having the class II state. The relation of pattern of these pale-pigmented areas to dose of active *Spm* elements is the same as that of the deeply pigmented areas. By observing these patterns in kernels having a class I state, therefore, it has been possible to conduct the same kinds of test as those described above, to distinguish between absence of *Spm* and its presence in an inactive phase.

In kernels having a class I state, then, two distinctly different types of variegated pattern appear: deeply pigmented areas, arising from mutation, which are solidly pigmented throughout; and pale areas, which reflect inactivations of *Spm*. When a pale area is large (one-*Spm* pattern) it usually contains smaller colorless areas within it, and some of these, in turn, may exhibit small specks of either the mutant phenotype or the pale phenotype. Mutant spots, it has been noted, always appear in a part of the kernel where *Spm* has been active in ancestor cells; they do not appear in the pale areas. In other words, mutations at a_2^{m-1} occur only in those cells in which *Spm* is in its active phase.

Examination of kernels carrying the class I states has also shown that the size of mutant spots is always small if activity of the *Spm* element commences late in

development. Thus, if a change in *Spm* from an inactive to an active phase is delayed until late in the development of a tissue, only small spots of mutant phenotype will appear in the areas in which *Spm* is active, even when the state that is present is known to be one that would produce many large areas of mutant phenotype, as a result of early-occurring mutations, if the *Spm* element were in its active phase during early development. Therefore, the size of a mutant spot produced by class I states of a_2^{m-1} is conditioned not only by the particular state itself but also by the timing of change in phase of activity of the *Spm* element.

Knowledge of the activity cycles of the *Spm* element in the a_2^{m-1} cultures has clarified many of the bewildering aspects of a_2^{m-1} behavior encountered early in its study. Its seemingly disorderly patterns of gene expression and apparently unorthodox types of inheritance behavior can now be interpreted and no longer give cause for confusion. The conditions responsible for change in activity of *Spm* are not yet understood, but studies are under way that may help elucidate them.

Before leaving this discussion, it may be useful to point out the resemblance between the patterns of pigmented spots, arising from inactivations of *Spm*, that appear with different doses of *Spm* and those that are produced by different doses of *Ac*. In the former case, the pattern is produced by change in phase of activity of *Spm*, whereas in the latter case it is produced by mutation-inducing events instigated by *Ds*, the complementary component of the *Ds-Ac* system. The resemblance of the two systems with respect to pattern type and dose effect is so great that it suggests some basic similarity in mode of operation.

*A Modifier Element in the a_1^{m-1} -*Spm* System*

When the standard *Spm* element of the a_1^{m-1} cultures is present, mutations occur

at the a_1^{m-1} locus in plants and kernels; the size and number of mutant areas reflect both the time of occurrence of mutation during development and the number of cells in which it takes place. As described earlier, the different states of a_1^{m-1} are recognized by the distinctive patterns of mutation they produce; and the expression of a state of a_1^{m-1} is not altered by dose of *Spm*, for the same pattern appears when one or more standard *Spm* elements are present.

During the course of study of a particular state of a_1^{m-1} , a single kernel appearing on one ear of a plant reflected a mutation frequency much higher than that expected of the state that was present in the plant. The plant grown from this kernel likewise exhibited a marked increase in mutation frequency. From testcrosses of this plant and examination of the progeny it became evident that a modifier element, independently located in the chromosome complement, was responsible for the increase in mutation frequency, and that the action of the modifier was expressed only in kernels and plants that also carried *Spm*. Further examination revealed that this modifier element underwent transposition. Preliminary findings about the modifier element were given in Year Book 56; and the investigation was continued this year, as reported below.

Effects of the modifier element on the expression of four different states of a_1^{m-1} were examined. When *Spm* is present, without the modifier, one of these states gives rise to a few, relatively late-occurring mutations; a second state produces more mutations, most of which occur relatively late in development; a third gives very many mutations, all occurring late in development; and the fourth produces many mutations, some of which occur early in development. It was found that the modifier alters the mutation patterns of only the first two of these four states; it does not change the patterns associated with the other two. In the two affected states, an

increase in frequency of mutations when the modifier is present is expressed in a stepwise manner.

The first-mentioned state, which gives relatively few mutations in the absence of the modifier, gives many more in its presence. The mutation pattern so produced resembles that of the second state when the modifier is absent. In turn, the effect of the modifier on the second state is to increase the mutation frequency so that the pattern of mutations resembles that of the third state described above.

In the testcrosses made with the first plant in which the modifier appeared, no linkage of the modifier with genetic markers in chromosomes 3, 5, or 9 was detected. In the progeny of this plant, however, it was early discovered that parts of some plants lacked the modifier and that other plants, or parts of plants, carried different numbers of modifier elements. Tests were conducted with a number of these plants in order to detect changes in location of the modifier that might place it in one of the three above-named chromosomes. It was detected in chromosome 3 in one plant, and in chromosome 9 in another. Tests were then carried out with some of the progeny of each of these two plants, to determine the constancy of location of the modifier.

One of the testcrosses made with a plant whose constitution was $a_1^{m-1} Sh_2/a_1 sh_2$ suggested that a modifier element was located in its $a_1 sh_2$ -carrying chromosome 3. The state of a_1^{m-1} in this plant was the second of the four described above. In another test, pollen of this plant was placed on the silks of a plant homozygous for the second state of a_1^{m-1} and for Sh_2 but carrying no *Spm* or modifier element. This cross produced 356 kernels, classified as follows: 1 deeply and uniformly pigmented (germinal mutation); 206 uniformly pale colored (no *Spm*); 65 variegated, with a number of small, deeply pigmented spots in a colorless background (*Spm*, no modifier); and 84 variegated,

with very many more mutant spots (*Spm* and modifier).

The silks of ears produced by 11 plants derived from kernels in the last category received pollen from plants homozygous for the second-described state of a_1^{m-1} and for sh_2 but having no *Spm* or modifier element. Eight of these 11 plants were $a_1^{m-1} Sh_2/a_1 sh_2$ in constitution, and the other 3 were homozygous for a_1^{m-1} and Sh_2 . The modifier element was present in all 8 plants having the former constitution; some plants had one modifier element and others had two, but each carried a modifier element in the $a_1 sh_2$ chromosome 3. In the cells that gave rise to the tested ears of 5 of these 8 plants, a single modifier element was present. A single *Spm* element was also present in these 5 plants, and was inherited independently of the modifier element (table 9, A). The

TABLE 9. *Spm* and Modifier Constitution of Different Plants of a Culture, as Determined by the Cross $a_1^{m-1} Sh_2/a_1 sh_2$, *Spm*, modifier ♀ × $a_1^{m-1} sh_2/a_1^{m-1} sh_2$, no *Spm*, no modifier ♂

Modifier Elements, location and number	<i>Spm</i> Elements, number	Plant No.	No. of Ears Tested per Plant
Group A			
$a_1^{m-1} Sh_2 +/a_1 sh_2 Mod$	1	2	1
		4	3
		5	1
		7	1
		8	1
Group B			
$a_1^{m-1} Sh_2 +/a_1 sh_2 Mod$, plus independently located modifier	1	1	1
		3	1
Group C			
Same as B	2	6	2

cells that produced the tested ears of 3 other plants had two modifier elements; one was located in the $a_1 sh_2$ -carrying chromosome, and the location of the other was not determined. The cells that gave rise to the tested ears of two of these plants contained a single *Spm* element (table 9, B), and those that produced the two tested ears of the third plant had two independently located *Spm* elements (table 9, C). The frequencies of the different types of kernels on the testcross ears, which revealed these

three types of constitution, are given in table 10. In A of table 10, the percentage of recombination between *sh*₂ and the modifier is 17.2. This value need not represent the percentage of crossing over between *sh*₂ and the modifier, for some of the kernels in the recombinant classes probably reflect the consequence of independent meiotic distribution of the modifier and *sh*₂ in some sporocytes in which the modifier had been transposed to a new location in an ancestor cell.

The modifier element located in chromosome 9 was detected when an ear of a plant of the constitution *a*₁^{*m-1*} *Sh*₂/*a*₁ *sh*₂,

in either chromosome 3 or chromosome 9. A modifier was present, located in the *Wx*-carrying chromosome, in at least some part of 6 of these plants. In the 7th (plant 5, table 11), the results of the testcross, made with the ear of the main stalk and two tiller ears, indicated that the cells producing each of these three ears had one modifier element, but that it was not located in chromosome 9, or, at any rate, not close enough to *Wx* to show evidence of linkage with it.

A similar situation was found with respect to the ear of the main stalk in plant 1, table 11, although tests of the tiller ear of

TABLE 10. Types of Kernels Appearing on Ears Produced by Plants Whose Constitutions Are Entered in A, B, and C of Table 9

Constitution	Phenotype of Kernel								Totals
	Deeply Pigmented (Germinal Mutation)		Uniformly Pale Colored (No <i>Spm</i>)		Spots of Deep Pigmentation in Colorless Background (<i>Spm</i> Present)				
					Few Spots (No <i>Mod</i>)		Many Spots (<i>Mod</i>)		
	<i>Sh</i> ₂	<i>sh</i> ₂	<i>Sh</i> ₂	<i>sh</i> ₂					
					<i>Sh</i> ₂	<i>sh</i> ₂	<i>Sh</i> ₂	<i>sh</i> ₂	
A	15	0	531	548	479	107	92	476	2248
B	5	0	193	166	67	21	89	141	682
C	4	0	78	89	95	21	112	192	591

Wx/wx was used in a cross with a plant homozygous for *a*₁^{*m-1*}, *Sh*₂, and *wx* and having no *Spm* or modifier element. The cells that gave rise to this ear had one *Spm* and also one modifier, the latter located in the *Wx*-carrying chromosome. Kernels from this ear that were *Wx* and showed the presence of both *Spm* and the modifier were used to grow 8 plants, which were tested for *Spm* number and for modifier number and location by means of crosses with plants homozygous for *a*₁^{*m-1*}, *sh*₂, and *wx* and having no *Spm* or modifier element. One of the 8 plants proved to have no *Spm* element, and therefore the presence of the modifier could not be detected by this cross. In all examined parts of the other 7 plants, however, one *Spm* element was present, and it was not linked with markers

the plant and also of the pollen of this tiller indicated that the modifier was linked with *Wx* in at least that part of the plant. In a tiller ear of plant 6, no modifier element was present; and absence of the modifier was also observed in part of a tiller ear of plant 2. In plant 2, the cells that gave rise to the main ear carried two modifier elements, one of which was linked with *Wx*. The distribution of kernel types derived from the testcrosses of plants 1 to 7 is given in table 12. Linkage of the modifier element with *Wx* is clearly expressed by the data of parts A and B of the table; but absence of such linkage is evident from the results of the tests of the three ears produced by plant 5 (part C). The types of kernels appearing on the tiller ear of plant 2 are recorded in part E.

TABLE 11. Number and Location of Modifier Elements in the Progeny of a Plant Having One Modifier Element Linked with *Wx* in Chromosome 9

Plant No.	Modifier Elements in Different Parts of Individual Plants			
	A <i>Wx Mod/wx</i> +	B <i>Wx Mod/wx</i> +; 1 <i>Mod</i> Independently Located	C <i>Wx/wx</i> ; 1 <i>Mod</i> Not Linked with <i>Wx</i>	D <i>Wx/wx</i> ; No <i>Mod</i>
1	Ear of tiller Pollen of tiller		Main ear	
2	Tiller ear *	Main ear		
3	Main ear			
4	Tiller ear			
5	Main ear		Main ear Ears of 2 tillers	
6	Main ear Pollen of main stalk			Tiller ear
7	Main ear Tiller ear			

* A sector of this ear had no modifier element. See part E, table 12.

TABLE 12. Types of Kernels Appearing on Ears Produced by Plants Whose Constitutions Are Entered in Columns A to D, Table 11

Constitu- tion Shown in Table 11, Column	Parent- age in Cross	Phenotype of Kernels								Totals
		Deeply Pigmented (Germinal Mutation)		Pale Colored (No <i>Spm</i>)		Spots of Deep Color in Colorless Background (<i>Spm</i> Present)				
						Few Spots (No <i>Mod</i>)		Many Spots (<i>Mod</i>)		
		<i>Wx</i>	<i>wx</i>	<i>Wx</i>	<i>wx</i>	<i>Wx</i>	<i>wx</i>	<i>Wx</i>	<i>wx</i>	
A	♀	14	17	498	490	150	388	364	109	2030
	♂	18	17	354	330	125	241	238	69	1392
B	♀	6	5	77	89	6	31	90	48	352
C	♀	2	5	215	229	99	119	98	102	869
D	♀	0	2	77	55	56	62	0	0	252
E *	♀	3	0	48	63	15	34	35	23	221
E †		0	1	17	19	29	22	0	0	88

* Tiller ear of plant 2; modifier present.

† Sector with modifier absent.

In one sector of this ear the modifier was absent. The cells producing the remainder of the ear carried a single modifier element, which appears to have been in the *Wx*-carrying chromosome, although the data are few and the expression of linkage is less marked than in the results entered in part A of the table.

The evidence outlined above, together with that obtained in other studies of the modifier, indicates that this element, like the elements *Spm*, *Ds*, *Ac*, and so forth, may undergo transposition from one location to another in the chromosome complement, at a time either early or late in the development of a plant. Another finding of the past year is that the expression of the modifier is the same whether the standard *Spm* or *Spm-w* is present. (For differences in effect produced by these two states of *Spm*, see Year Book 56.) In the absence of the modifier, on the other hand, the distinct pattern of mutation at a_1^{m-1} effected by each of these two states of *Spm* is clearly expressed. The difference may be observed among the kernels on ears of plants carrying both states of *Spm* as well as the modifier, when such ears are used in the testcross described above. Those kernels having one or the other state of *Spm* but no modifier are readily distinguished from each other, whereas kernels with either state of *Spm* and also the modifier cannot be distinguished from one another. Thus, the modifier element appears to be a component of the *Spm* system that can modify the expression both of a state of a_1^{m-1} and of the *Spm* element.

Continued Investigation of Transposition of Spm

A report was given in Year Book 56 of two progeny tests made to determine the time and frequency of transposition of the standard *Spm* element carried in the a_1^{m-1} cultures. The ear-producing parent in one of these tests had one *Spm*, located in one of its chromosomes 9. The numbers of

Spm elements present in different plants of the progeny, and in different parts of a single plant, and also the location of *Spm* with reference to the marker *Wx*, carried in chromosome 9, were summarized in table 6, page 394, Year Book 56. The tests reported there were extended this year, with plants derived from variegated, *Wx* kernels on five of the ears recorded in that table: the second and tiller ears of plant 7285A-1, a tiller ear of plant A-2, a tiller ear of plant A-7, and the main ear of plant B-6. The cells that gave rise to four of these ears had one *Spm* element, located in the *Wx*-carrying chromosome 9. The cells that gave rise to the fifth ear (tiller ear of plant A-1) carried one *Spm*; but it was not linked with *Wx*, even though testcrosses of both the first and second ears of the main stalk of this plant showed linkage of *Spm* with *Wx*. The plants studied last year provided a number of examples of transposition of *Spm* occurring early in development. The tests conducted this year were made for two purposes: first, to learn whether or not early-occurring transposition of *Spm* would continue to be expressed in the progeny plants; and second, to learn whether or not the transposition of *Spm* away from a known location in chromosome 9 might be followed later by transposition back to its former location.

To carry out the first of these purposes, *Spm* constitution and location were determined in 37 progeny plants. Each plant was grown from a variegated, *Wx* kernel on an ear produced from cells in which *Spm* had been located in the *Wx*-carrying chromosome 9. Twelve plants were derived from one ear, 8 plants each from two other ears, and 9 plants from a fourth ear. Table 13 summarizes the results of these tests, which have been incorporated into a single table because early-occurring transposition of *Spm* was observed among the plants of all four cultures. It may be seen from the table that early-occurring transposition of *Spm*, evident in the parent

plants, was likewise exhibited in their progeny.

To determine whether or not sequential transpositions of *Spm* may be directed in such a way that removal of *Spm* from a

mentioned above. In this tiller, the *Spm* element had been transposed away from a location close to *Wx* in chromosome 9. Twenty-one ears in all were obtained from these 11 plants. Among them, there were

TABLE 13. *Spm* Constitution and Location in Different Plants and in Different Parts of Individual Plants

No. of Ears Tested per Plant	Position of Ear in Plant	No. of Plants	<i>Spm</i> Constitution and Linkage with <i>Wx</i>	No. of Plants with Given Constitution
1	1st ear, main stalk	13	1 <i>Spm</i> , linked with <i>Wx</i> 2 <i>Spm</i> , neither linked with <i>Wx</i> No <i>Spm</i>	9 * 3 1
2	1st and 2nd ear, main stalk	1	1 <i>Spm</i> , linked with <i>Wx</i>	1
2	1st ear, main stalk Tiller ear	15	1 <i>Spm</i> , linked with <i>Wx</i> , in both ears 1 <i>Spm</i> , linked with <i>Wx</i> , in 1st ear; 1 <i>Spm</i> , not linked with <i>Wx</i> , in tiller 1 <i>Spm</i> , linked with <i>Wx</i> , in 1st ear; 2 <i>Spm</i> , one linked with <i>Wx</i> , in tiller 2 <i>Spm</i> , one linked with <i>Wx</i> , in both ears 1 <i>Spm</i> , not linked with <i>Wx</i> , in both ears	10 1 1 1 1 2
3	1st and 2nd ear, main stalk Tiller ear	6	1 <i>Spm</i> , linked with <i>Wx</i> , in all three ears 1 <i>Spm</i> , linked with <i>Wx</i> , in 1st and 2nd ears, main stalk; 2 <i>Spm</i> , neither linked with <i>Wx</i> , in tiller 2 <i>Spm</i> , one linked with <i>Wx</i> , in 1st ear; 1 <i>Spm</i> , linked with <i>Wx</i> , in 2nd ear; no <i>Spm</i> in tiller 2 <i>Spm</i> , one linked with <i>Wx</i> , the other linked with <i>Pr</i> , in all three ears	3 1 1 1
3	1st ear, main stalk Ear on each of two tillers	2	1 <i>Spm</i> , linked with <i>Wx</i> , in all three ears	2

* One ear with sector in which *Spm* was absent.

known location in chromosome 9 may be followed by a return to that location, tests of *Spm* number and location in different plants and different parts of the same plant were conducted with 11 plants derived from the variegated, *Wx* category of kernels on the tiller ear of plant 7285A-1,

six certain cases of successive transpositions of *Spm*; but in none of the ears was *Spm* found to be linked with *Wx*. Although this test is limited, it does indicate that a directed type of transposition of *Spm*, which returns it to the location it previously occupied, does not occur regularly.

BIBLIOGRAPHY

- Campbell, A. Division synchronization in a respiratory deficient yeast. *J. Bacteriol.*, 74, 559-563 (1957).
- Campbell, A. Effect of starvation for glucose during reversion of a long term adapting yeast. *J. Bacteriol.*, 74, 553-558 (1957).
- Campbell, A. Synchronization of cell division. *Bacteriol. Rev.*, 21, 263-272 (1957).
- Campbell, A. Transduction and segregation in *Escherichia coli* K12. *Virology*, 4, 366-384 (1957).
- Campbell, A. The different kinds of transducing particles in the λ -gal system. *Cold Spring Harbor Symposia Quant. Biol.*, 23, 83-84 (1958).
- Clowes, R. C. Investigation of the genetics of cysteineless mutants of *Salmonella typhimurium* by transduction. *J. Gen. Microbiol.*, 18, 154-172 (1958).
- Clowes, R. C. Nutritional studies of cysteineless mutants of *Salmonella typhimurium*. *J. Gen. Microbiol.*, 18, 140-153 (1958).
- De, D. N. Pollen tube mitosis without culture medium: metaphase arrest by acenaphthene. *Stain Technol.*, 33, 57-58 (1958).
- Demerec, M. Structure and arrangement of gene loci. In *Cytologia*, Suppl. Vol., *Proc. Intern. Genet. Symposia*, pp. 20-31, 1956.
- Demerec, M. Genetic aspects of drug resistance. In *Ciba Foundation Symposium on Drug Resistance in Micro-Organisms*, pp. 47-58, 1957.
- Demerec, M. (Editor). *Advances in Genet.*, 9, 294 pp., 1958.
- Demerec, M., I. Goldman, and E. L. Lahr. Genetic recombination by transduction in *Salmonella*. *Cold Spring Harbor Symposia Quant. Biol.*, 23, 59-68 (1958).
- Gay, H. Review of *Intern. Rev. Cytol.*, vol. 6. *Science*, 127, 869 (1958).
- Gay, H. See also Kaufmann, B. P.
- Goldman, I. See Demerec, M.
- Hershey, A. D. Experimental problems concerning the role of deoxyribonucleic acid in growth of bacteriophage T2. *Cellular Biology, Nucleic Acids, and Viruses, Special Publ. N. Y. Acad. Sci.*, 5, 251-257 (1957).
- Hershey, A. D. Some minor components of bacteriophage T2 particles. *Virology*, 4, 237-264 (1957).
- Hershey, A. D. The production of recombinants in phage crosses. *Cold Spring Harbor Symposia Quant. Biol.*, 23, 19-46 (1958).
- Howarth, S. Suppressor mutations in some cystine-requiring mutants of *Salmonella typhimurium*. *Genetics*, 43, 404-418 (1958).
- Käfer, E. An 8-chromosome map of *Aspergillus nidulans*. *Advances in Genet.*, 9, 105-145 (1958).
- Kaufmann, B. P., and H. Gay. The nuclear membrane as an intermediary in gene-controlled reactions. *The Nucleus*, 1, 57-74 (1958).
- Lahr, E. L. See Demerec, M.
- Moser, H. *The Dynamics of Bacterial Populations Maintained in the Chemostat*. Carnegie Inst. Wash. Publ. 614, 136 pp., 1958.

PERSONNEL

Year Ended August 31, 1958

- Balbinder, Elias, Associate in Research
- Balbinder, Evelyn (Mrs.), Research Assistant
- *Banič, Stanko, Fellow of Boris Kidrich Foundation, Yugoslavia
- Bennett, James L., Maintenance Man
- Buchanan, Jennie S. (Mrs.), Research Assistant
- Burch, Barbara J. (Mrs.), Stenographer
- *Campbell, Allan M., Associate in Research
- Carley, Catherine, Switchboard Operator and Computer
- Catcheside, David G., Carnegie Institution Fellow
- Datta, Mihir Kumar, Research Assistant
- Demerec, M., Director
- Fisher, Agnes C., Secretary to Director
- *Fredericq, Pierre, Fellow of the Belgian American Educational Foundation, Inc.
- Gay, Helen, Associate in Research
- Glanville, Edward, Research Assistant
- *Goldman, Irving, Research Assistant
- *Gross, Julian D., Research Assistant
- *Haggerty, Michael J., Maintenance Man
- *Hashimoto, Kazuo, Carnegie Institution Fellow
- Hershey, Alfred D., Microbiologist
- Jones, Henry H., Photographer
- Karossa, Judith, Research Assistant
- Kaufmann, Berwind P., Cytogeneticist
- Koch, Gebhard, Associate in Research
- Lahr, Ernest L., Associate in Research
- McClintock, Barbara, Cytogeneticist
- McDonald, Joseph L., Janitor
- McDonald, Margaret R., Chemist

* Resigned during the year.

McDonald, William T., Janitor
 McIntyre, Jean W. (Mrs.), Technical Assistant
 Mandell, Joseph D., Associate in Research
 Meissner, Richard C., Superintendent of Buildings and Grounds
 Miyake, Tadashi, Research Assistant
 Peckham, Leslie E., Senior Clerk
 Rogers, Claude F., Chief Clerk
 Sepe, Domenico, Greenhouse Man
 Smith, Guinevere C. (Mrs.), Librarian, Curator of *Drosophila* Stocks
 Snyder, Emmy M. (Mrs.), Technical Assistant
 ‡Streisinger, George, Associate Geneticist
 Thomas, René, Rockefeller Foundation Fellow
 Tomizawa, Jun-ichi, Associate in Research
 Van Houten, William B., Engineer
 Westergaard, Mogens, Carnegie Institution Fellow
 White, Harry, Chief Mechanic
 Wilson, Carole E., Technical Assistant
 *Yoshida, Yoko, Research Assistant

Summer 1958 and Temporary

Bert, Grace R., Assistant
 Burtch, Ethel P. (Mrs.), Typist
 Cannon, W. Dilworth, Jr., Assistant
 Chao, Anna, Assistant
 Clark, Richard D., Maintenance Man
 Denker, Henry, Maintenance Man
 Fuscaldo, Kathryn E., Assistant
 Hartman, Philip E., Guest Investigator

‡ On leave of absence, September 1957 to September 1958.

Nevole, Florence, Assistant
 Nevole, Nancy A., Assistant
 Pederson-Krag, Gillian, Assistant
 Reinhold, Robert, Assistant
 Saunders, John L., Maintenance Man
 Šerman, Draško, Guest Assistant
 Simrell, Elizabeth Jane, Assistant to Librarian
 Staber, Elaine, Assistant
 Victoria, William, Maintenance Man
 Warhurst, Derek I., Maintenance Man

Collaborators at Biological Laboratory

Caspari, E. W., Summer Investigator
 Cicchetti, Joan, Stenographer
 *Englesberg, E., Staff Geneticist
 Franzese, Eleanor, Business Manager
 Goodgal, S. H., Summer Investigator
 Gots, J. S., Summer Investigator
 Hyde, Olive, Administrative Assistant
 Kaudewitz, F., Summer Investigator
 Kikkawa, H., Summer Investigator
 King, J. C., Staff Geneticist
 Leupold, U., Summer Investigator
 Luria, S. E., Summer Investigator
 Moser, H., Staff Geneticist
 Oksala, T., Summer Investigator
 Roper, J. A., Summer Investigator
 Sams, Joan, Research Assistant
 Schalet, A., Research Associate
 Slizynska, H., Summer Investigator
 Slizynski, B. M., Summer Investigator
 Vogel, H., Summer Investigator
 *Wallace, Bruce, Staff Geneticist
 von Wettstein, D., Summer Investigator
 Witkin, E. M., Summer Investigator

DEPARTMENT OF ARCHAEOLOGY

Cambridge, Massachusetts

H. E. D. Pollock, *Director*

CONTENTS

	<i>page</i>
<i>The Past</i>	435
<i>The Present</i>	448
<i>Ceramic Studies in Yucatan</i>	450
<i>Art and Artifacts</i>	451
<i>Ceramic Technology</i>	451
<i>Publications</i>	454
<i>Bibliography</i>	455
<i>Personnel</i>	455

THE PAST

In this final report it seems appropriate to review the past activities of Carnegie Institution of Washington in anthropology and archaeology. This is a history of over fifty years of research, the results of which fill something like one hundred volumes of Institution publications and of course many papers published elsewhere. It is clear, then, that in the space of the next few pages we must view this great body of work from a distance, omitting all detail, seeking out trends and stages of development in research, to the loss, unfortunately, of many a fine discovery, and to the further loss of much of the flavor and excitement of the work.

Upon the incorporation of Carnegie Institution, early in 1902, the Executive Committee, acting under instructions of the Trustees to determine what work should be undertaken, proceeded to appoint a number of Advisory Committees. Eighteen such committees, covering areas in the humanities, the sciences, and the social sciences, were named. One of these was for anthropology. It consisted of W. H. Holmes, Franz Boas, and G. A. Dorsey. It would have been difficult, if not impossible, to assemble three more celebrated anthropologists.

The report of this committee, published in Year Book 1 (pp. 174-181), is not without interest as we view it from the vantage point of fifty years. One senses that these men were uneasy in being called upon to review so vast a subject and to compress their findings in the space of a few pages. Touching upon the broad field of anthropology, the subjects that come under that discipline, and the work then being carried on in the Americas, the report moves on to suggestions for work by the Institution. Here the subject is treated under the then standard divisions in America: physical anthropology, archaeology, and ethnology. Although the desirability of knowledge of the cultures of

Africa, Asia, and the Pacific Islands was noted earlier in the report, all recommendations were for work in the United States. In the light of developments that were to come a decade later, we should like to quote one sentence from the discussion leading to recommendations for work in archaeology: "In middle and South America much is still to be done, but your committee has only been able to find, and that at the last moment, one person properly qualified for this branch of research."

Of the three projects recommended by the committee's report, two, namely, in archaeology and ethnology, were promptly instituted. For the next two years Holmes carried on a search for geologically early remains of man in America; Dorsey concerned himself with the ethnology of the American Indian for the following five years. We cannot refrain from noting that being "in on the ground floor" was not without its advantages, and that a sort of nepotism within committee was not below such giants as Holmes and Dorsey.

In spite of the fact that the Advisory Committee on Anthropology had made no specific recommendation for work outside the United States, research in Old World archaeology promptly got under way. In response to a commission by the Institution, a rather full report on the opportunities for archaeological researches in the lands of the eastern Mediterranean appeared in Year Book 2 (pp. 213-242). Even before this report was prepared, however, two projects concerned with the archaeology of the Near East had begun. One of them, the expeditions of Raphael Pumpelly to Turkestan and his excavations at Anau, carried on in 1903 and 1904, stands not only as a high point in the researches fostered by Carnegie Institution but as a shining light in the archaeology of the Near East. Properly controlled excavation and detailed recording rolled back the centuries to times previously un-

dreamed of, and with the two-volume report (C. I. W. Publ. 73) that followed, the prehistoric archaeology of the Near East left the realm of fancy and entered the more rigorous climate of fact.

With the exception of Pumpelly's expeditions in Turkestan, researches in anthropology and archaeology during the Institution's first decade seem to have been a matter of not infrequent but relatively small grants to individuals. Researches were wide-ranging in area and in subject. The ethnology of the American Indian and the search for geologically early man in America have been mentioned. Other studies involved Oriental art, Near Eastern, Egyptian, and Nubian archaeology, Classical archaeology at Rome and Athens, and archaeology and linguistics in Polynesia. It seems to have been a period of supporting the competent individual interested in worth-while research. There appears to have been no coherence among the various projects, no over-all plan as to what direction archaeology and anthropology might take under Institution auspices. Projects tended to run for about five years, but with no set term. An exception was the work of E. B. Van Deman in Roman archaeology, which continued for many years.

In 1913 the Institution published *Reports upon the Present Condition and Future Needs of the Science of Anthropology* (Publ. 200), which consisted of papers by W. H. R. Rivers, A. E. Jenks, and S. G. Morley that not only reviewed the status of anthropology at that time but also made recommendations for future work. It is interesting to speculate as to which of the three plans of research presented in those reports would today be considered the most stimulating, potentially the most productive of results, and the most suitable for undertaking by Carnegie Institution. Whatever the choice might now be, Morley's project, which called for intensive archaeological research at the Maya ruins of Chichen Itza in Yucatan, was approved by the Trustees, who, in so doing, pretty well set the course of the Institution's

work in anthropology and archaeology for the next forty-five years. With the exception of Van Deman's researches in Roman archaeology, and a few sporadic projects, notably one dealing with Kamchatka and the Aleutian Islands, all other work in anthropology was terminated and for the next fifteen years attention was directed almost solely to the archaeology of the Maya.

The following year, 1914, Morley was appointed Research Associate in American Archaeology, a title that was shortly changed to Associate in American Archaeology. Although his plan had called for intensive work at Chichen Itza, and that to be done on a sizable scale, the project was not to be put in operation for another decade. It will be remembered that this was the revolutionary period in Mexico, and conditions were not auspicious for establishing a long-term contract with the government of that country or the initiating of large-scale operations. It is further possible that the Institution felt some stringency of available funds and a lack of available and competent staff for the project. At all events, research in Maya archaeology was supported by a series of minor grants until 1921 or 1922, when the work reached a scale demanding support by large grants.

The time for intensive work at the ruins of Chichen Itza not being ripe in 1914, Morley suggested, and received approval for, "a work containing descriptions and decipherments of all known Maya texts." Hieroglyphic research was the side of Maya archaeology nearest to Morley's heart, and remained so throughout his life. Not content with the then known body of glyphic material, he soon proposed to the Institution, and again received approval, that further field work be undertaken. For the next half dozen years, sometimes alone, sometimes with one or two companions, Morley conducted extensive, indeed indefatigable, explorations, often under the most trying and even dangerous conditions. His travels took him time and

again into the jungles of the Peten region of Guatemala, time and again to the great ruins of Copan, down the Chamelecon River valley in Honduras, along the eastern coasts of Honduras and Nicaragua, to Costa Rica, and to Yucatan. New sites were discovered, and many new hieroglyphic inscriptions were recovered. An event that was to have considerable effect on the future work of the Institution in archaeology should be mentioned, namely the discovery in 1916 of the ruins of Uaxactun with "the oldest monument yet reported from the Maya field." In 1920 appeared Morley's huge monograph *The Inscriptions at Copan* (Publ. 219), it being realized at that time that the corpus of Maya hieroglyphic inscriptions had grown so large, and knowledge concerning them so extensive, that the original idea of publishing all known Maya texts in a single work was not practicable.

The Institution's interest in Maya archaeology had grown by 1921 to require the annual appropriation of a large grant. Although the nature of the research did not materially change for the next three years, the scope was expanded. Limited excavations were undertaken, and archaeology was supplemented by brief studies in linguistics and agronomy. In 1922 the President notes (Year Book 21, p. 13), "It is also of much importance to us in the near future to expand considerably our program of studies in the field of Middle American Archaeology." In 1923 a five-year permit, supplanted in 1925 by a contract of similar term but with the option of renewal for another five years, for archaeological research in Guatemala was obtained from the government of that country. A ten-year contract was entered into with the government of Mexico. The way was now prepared for the larger and different type of operations that the Institution was about to undertake.

This second decade, 1914-1923, of anthropological research may be characterized somewhat as follows. In the first place the Institution had set itself squarely

in the field of Middle American archaeology and had put aside nearly all other anthropological activities. The nature of the work was primarily exploration and hieroglyphic research. A number of new sites and many new monuments with hieroglyphic texts had been discovered. The broad outlines of Maya history as conceived by Morley in 1913 had not substantially changed during the decade, but detail had been added, and a very considerable body of new material had become available for future studies. The first volume of Morley's great work on "descriptions and decipherments of all known Maya texts" had appeared, and a site report and archaeological area study (Publ. 335) was in press.

It is not surprising that in the ten years that had intervened since Morley presented his plan calling for intensive work at the ruins of Chichen Itza his ideas as to what would constitute an adequate attack on the problems of Maya prehistory had changed. In the Institution's original permit from the Guatemalan government permission was granted for excavations at Uaxactun, Piedras Negras, and Tayasal, in the Department of Peten. The final contract omitted mention of Piedras Negras but included exploratory privileges at all other sites in the Peten. The contract with the Mexican government specifically named Chichen Itza. Morley's purpose in selecting these three sites (Year Book 25, pp. 273-274) was to bring under study the entire range of Maya history.

Two principles, that if not unique were at least novel at that time, were set forth in Morley's 1913 plan. The first was that the Institution, cooperating wholeheartedly with the antiquity laws of the country, should make no effort, by permission or otherwise, to export any objects discovered, but should return them all to the appropriate governmental authority once their study was completed. The second was that the excavator had an obligation to preserve the remains uncovered from further deterioration. Reconstruction of architec-

tural remains was specifically mentioned. Undoubtedly this farsighted policy was instrumental in our gaining contracts with governments that at that time were far from reassured about *gringo* motives, and was further instrumental in maintaining extraordinarily cordial relations with those governments. It also set a standard for other organizations practicing archaeology in Middle America.

Morley's dream of intensive archaeological work at Chichen Itza became a reality in 1924. Large-scale excavations, followed in due time by consolidation and restoration of buildings, began that year and continued almost uninterruptedly for the next decade. A dozen or more structures were rebuilt or repaired to varying stages of completeness. Many more were tested by means of minor excavations, and scores were examined for all that could be learned from what was visible above ground. At the ruins of Uaxactun, after a preliminary survey of the site in 1924, intensive work got under way in 1926 and continued for a dozen years. One huge complex of related buildings was completely excavated, several lesser structures were similarly treated, and many others were uncovered to varying extent. Parenthetically, the contemplated work at Tayasal, where preliminary excavations had been carried on in 1921 and 1922, was never undertaken. Along with the large-scale excavations at Chichen Itza and Uaxactun, exploration elsewhere in the Maya area continued. Year after year expeditions pushed into the little-known regions of British Honduras, the Peten, the Usumacinta drainage, Campeche, Yucatan, and Quintana Roo. New sites were discovered; previously known sites were newly explored; many new hieroglyphic texts were recorded. Morley's hieroglyphic research continued unabated.

Although the work outlined above continued well beyond 1930, quite a different program of operations began in that year, and it seems well to review the period 1924-1929 before moving on. By and large

these years were characterized by intensive excavation, restoration of large architectural remains, exploration, and hieroglyphic research. Excavation and restoration on any such scale were new to Maya archaeology and virtually unparalleled throughout Middle America. Equally new, although far less dramatic, were the beginnings of the systematic study of the lesser artifacts of the Maya. Previous to this time Maya studies had been concerned primarily with the hieroglyphic texts, the documentary history, and the spectacular art and architecture. Earlier excavations had turned up pottery and implements of stone, but these had gone largely unstudied. In 1926 the late George C. Vaillant undertook the study of pottery remains collected under stratigraphic controls at Chichen Itza, and in the following year he produced a doctoral thesis dealing with the chronological significance of Maya ceramics. Good-sized excavations were now under way at Uaxactun, and pottery was appearing in quantity. In 1928 Vaillant visited Uaxactun, and in the few days he was there sank a small pit through successive plaza floors and into the underlying earth. The pottery that came up was not Maya in the sense of what had until then been thought of as Maya pottery; it was akin to the early Archaic pottery of the Mexican and Guatemalan highlands. It was, moreover, obviously earlier than the structural remains above, which in turned were considered to be the earliest discovered up to that time. A whole new horizon of Maya prehistory appeared in the bottom of that pit, and the systematic study of ceramic remains was well on its way.

Clearly, the Institution's interest in archaeology at this time was active and was growing. Indeed, it was not confined to archaeology but included the whole history of man and his works. In 1923 the President wrote (Year Book 22, pp. 10-11):

The plan of study of Chichen Itza concerns the broader problem of early American

history as it can be interpreted through the Maya civilization. Along with specifically archaeological investigations touching the history of engineering, architecture, art, and the stratigraphic sequence of cultures, the researches will include a study of the physical characters of the race and of the environment in which it developed. In order to understand these people as they lived and to secure information concerning their industries and their agriculture, it is necessary to know the limitations imposed by geological, climatological, and other physical conditions determining the development of the plants and animals upon which the inhabitants were dependent. The studies proposed will naturally require the assistance of a considerable group of specialists and it is hoped that through cooperation of other agencies and institutions interested in this work a thoroughly fundamental investigation may be carried out.

The broad study outlined in that paragraph was not to come for some years, but it apparently was held steadily in mind. The person who finally was to implement the program was A. V. Kidder. In 1926 Kidder, who had earlier been retained in the capacity of adviser on the archaeological work of the Institution, was appointed Research Associate, and the following year Associate in charge of archaeology. In 1929 he was named Chairman of the newly formed Division of Historical Research. The Division, which was an administrative grouping of most of the humanistic studies being carried on by the Institution, consisted of the Section of Aboriginal American History, Section of United States History, Section of the History of Science, and Associated Investigations in Palaeography and in the History of Greek Thought. This organization of the Division was to change over the years, notably by the termination in 1936 of the Section of United States History and the substitution in its place of the Section of Post-Columbian History. As our prime concern here is the work in archaeology and anthropology, we shall follow the course only of studies that in one way or

another relate to the history and culture of aboriginal man.

Kidder immediately set about organizing the many-sided approach to the study of man that had been envisioned by the President. In 1930, the first year of operation under the new Division, there were either in operation or in process of organization studies in archaeology, epigraphy, physical anthropology, medicine, social anthropology, linguistics, aboriginal documentary history, colonial history, and environmental studies involving plant and animal biology. To this list, over the years, were added studies in ceramic technology, geography, geology, and agronomy. A good part of the work was cooperative in nature, notably with the University of Chicago and the University of Michigan. The central effort focused upon Middle America, and particularly the Maya area, but other work, primarily archaeological, was carried on in the Southwest of the United States.

Even to list the many activities, not to mention their results, under this widespread program of research would go beyond the limits of our report. The largest effort in man power and resources went into Middle American archaeology, and this part of the work, which will be mentioned again, came nearest to acting as a unifying force in so varied a program. During the dozen years from 1930 to the entry of the United States into the war, certain parts of the program operated virtually continuously. This was of course true of the work in archaeology, both Middle American and Southwestern. It was also true of the work in social anthropology and the linguistic studies, which dealt with both lowland and highland Maya peoples. Studies in the aboriginal documentary history of the Maya and the colonial history of the Maya area, which were begun in 1931, also continued throughout this period. Research in ceramic technology, begun in 1934, went on without interruption. Work in physical

anthropology, medicine, and the environmental studies of biology, geography, geology, and agronomy were carried on for shorter lengths of time. There are excellent reviews of this work as it progressed in Kidder's annual reports during that period (Year Books 29-40), notably in Year Book 38 (pp. 235-240). Here we shall turn to an account of the archaeological researches.

Before taking up the Institution's central effort in the Maya area it should be remarked that near the beginning of the period we are now reviewing the Institution entered a new archaeological field, namely, the Southwest of the United States. This work was carried on by the late E. H. Morris, who had been in charge of excavations at Chichen Itza from 1924 to 1928. The field work centered in the northern parts of Arizona and New Mexico and southern Colorado. These researches, which continued uninterruptedly until Morris's retirement in 1955, were never a large effort compared with that in Middle America, but nevertheless were highly productive. Particularly worthy of mention is Morris's elaboration of our knowledge of the early Basketmaker II period of Pueblo culture, and his discovery of remains that, through dendrochronological methods, allowed the chronology of the northern Pueblo area to be carried back to a time before the Christian Era.

Returning to the archaeological program in Middle America, it has already been mentioned that intensive work continued at Chichen Itza and at Uaxactun until the mid 1930's. With the tapering off of activities at Chichen Itza, a somewhat similar project, jointly sponsored by the Institution and the Honduranian government, and involving excavation and stabilization of buildings and monuments, was instituted at Copan. There was also a small project for the repair and stabilization of monuments at the ruins of Quirigua in Guatemala. As has been mentioned above, stratigraphic methods of excavation, particularly applied to pottery and other lesser

artifacts and especially fruitful in connection with the work at Uaxactun, were under way. Activities of this sort were likewise carried on in British Honduras. The importance of this work cannot be overstressed. From Uaxactun emerged a chronological sequence of architectural styles and pottery types (C. I. W. Publ. 588, and Mid. Amer. Res. Inst., Tulane Univ., Publ. 20), the latter ably reinforced by the work in British Honduras, that is fundamental to our knowledge of Classic lowland Maya culture.

Exploration, continuing actively, involved not only individual sites but surveys of sizable areas, notable examples of the latter being expeditions into the hill regions of Yucatan and Campeche and to southern Campeche and southern Quintana Roo. Work of this sort extended as far as southern Veracruz, where a cooperative effort with the Mexican government was undertaken. Hieroglyphic research, always the primary interest of Morley, was now reinforced by the unfortunately brief but brilliant work of J. E. Teeple, and the equally brilliant and long-continuing work of J. E. S. Thompson. Last, there were studies concerned with the simple, domestic houses and the living patterns of the people, aspects of Maya culture that had largely been disregarded until this time. As will appear in subsequent pages, these studies foreshadowed the work of the Department in later years.

So far we have been referring to researches restricted for the most part to the Maya lowlands. Almost by definition Morley looked upon Maya civilization as a lowland phenomenon. That this position might be open to question was suggested by the knowledge that Maya-speaking peoples had inhabited the Guatemala and Chiapas highlands at least since the Spanish conquest and the suspicion that they had been there for many centuries before that event. At Uaxactun the deeply buried ceramic remains, whether considered to be Maya or not, intimated highland-lowland connections at an early time. At all

events, in 1930 we find Kidder raising the question whether it might be desirable to undertake excavations in the Guatemala highlands. A more definite statement to this effect by the late O. G. Ricketson appeared the following year, and archaeological survey and excavation began in 1932. With these gradual and rather tentative moves the Institution broadened its archaeological program to include the Maya highlands, an area that was at the same time being brought under study by a number of other disciplines in the overall program of the Division of Historical Research.

Archaeological researches in the highlands, which continued almost without interruption for approximately twenty years, included both excavation and survey. A good part of the work centered in the great ruin site of Kaminaljuyu, lying on the outskirts of Guatemala City. The choice of this site was in part planned, in part fortuitous, owing to modern building operations and public works incidental to an expanding Guatemala City. Important archaeological remains were being uncovered willy-nilly, and there was pressure on the Division's staff from the local governmental office in charge of archaeology, as well as the urgings of professional responsibility, to see that knowledge of such remains was not forever lost. Indeed, it was uncontrolled digging by local people that led to the Institution's undertaking an operation that stands as a milestone in Middle American archaeology. The work of Kidder, Jennings, and Shook (Publ. 561) in 1936, 1937, 1941, and 1942 at two inconspicuous mounds at Kaminaljuyu resulted in the formulation of a time-space network that linked the great Classic centers of Middle American culture in the Valley of Mexico, the Valley of Oaxaca, the Guatemala highlands, and the Peten lowlands. Not only were breached the artificial boundaries that had seemed to surround lowland Maya civilization, but for the first time the high cul-

tures of Middle America came into focus in time and space as parts of one great area of relatively homogeneous, or at least interdependent, civilizations. It is hardly possible to exaggerate the effect of these discoveries on Middle American archaeology.

Inasmuch as the entry of the United States into war late in 1941 severely disrupted the normal activities of the Institution, the archaeological and allied researches being no exceptions, it seems well to pause here in an effort to characterize the work of the dozen years from 1930 through 1941. Certainly the epitome, the very essence, of the program was its many-sided approach to the study of man. Time and again in annual reports to the Institution and in papers published elsewhere Kidder presented the rationale and the philosophy underlying that approach. They may be stated somewhat as follows: that human history is a continuum, beginning in the unknown past and still in the making; that man's actions and his culture are intelligible only when focused against his environment; that the story of man can be recovered and properly told only through the aid of researches under various disciplines. To attempt even to summarize the results of this broad program would exceed the limits of the present report. Kidder has gone a considerable way in doing this, unfortunately in an unpublished report, and Morley (*Proc. Am. Philosophical Soc.*, 86: 205-219) has treated the more strictly archaeological activities. Here we must content ourselves with brief comments on the work directly related to anthropology and archaeology.

As mentioned earlier, research in social anthropology was carried on in the Maya lowlands and the highlands. It started in Yucatan, and by the end of the period we are reviewing several volumes and a number of papers covering the results of the work had appeared. Little had been published up to that time on the Guatemala highland studies, but a sizable amount of

material had been amassed that was to appear later, although not under Carnegie Institution imprint. Even though most of this work with modern Maya culture was not historically oriented, and its connections with the central historical and archaeological program were at the time somewhat tenuous, it did point out problems that were subject to, and that since have been brought under, archaeological investigation.

Linguistic research, which had gone on since the beginning of the period and had covered an extraordinarily wide range of Mayan-speaking peoples, was abruptly terminated, temporarily, by the untimely death in 1941 of M. J. Andrade, who had been in charge of that part of the program. Andrade left extensive records, including a nearly completed manuscript of a Yucatec grammar, but virtually nothing had been published.

Studies in physical anthropology were never a large part of the program. Some work had been done on the living Maya of Yucatan, resulting in two publications. There were also the descriptions in archaeological reports of the none too plentiful human skeletal remains produced by excavations.

Research in aboriginal documentary history, primarily concerned with pre-conquest times in contrast to colonial history and hence more directly applicable to archaeological researches, was highly productive. By the end of the period we are considering, two translations of important Yucatecan Maya documents, magnificently annotated, had appeared, and a third, more general volume (Publ. 548) dealing with the history of Yucatan at the time of the Spanish conquest appeared two years later. This work, which was of particular value to hieroglyphic research, did much to advance our understanding of the social, religious, and intellectual content of Maya culture, knowledge of a sort that is so difficult to wring from the material remains of a past civilization.

It has previously been mentioned that the hieroglyphic researches of Morley were amplified at this time by the efforts of Teeple and of Thompson. The work of these men resulted in a fundamental change in prevalent reconstructions of Maya history. The Morley-Spinden correlation of the Maya and Christian calendars, the most widely accepted correlation up to that time, was quite generally abandoned in favor of the later Goodman-Martinez-Thompson correlation. The effect of this change, which tended to be confirmed by certain archaeological discoveries, was to place the beginnings of Classic Maya civilization around A. D. 300 instead of near the start of the Christian Era. This led to surrendering the long-held idea that the northern Yucatan development of Maya civilization, the so-called New Empire, came about only after the abandonment of the great southern centers, located in the so-called Old Empire, and substituted the idea that the northern cities were at least in part coeval with the Old Empire. During this period Thompson was busily working with, and publishing papers on, new interpretations of hieroglyphs, work that was to bear even heavier fruit in later years. In 1938 appeared Morley's great five-volume compendium, *The Inscriptions of Peten* (Publ. 437), the second major contribution to his lifetime task of compiling "a work containing descriptions and decipherments of all known Maya texts."

We have already touched upon the general course of the archaeological work during these years. Here we should like to point to trends that characterized the period and to certain fundamental advances in our knowledge of Maya civilization. It will be remembered that intensive excavation and the restoration of architectural remains had been continuing much as in the previous decade. As the period progressed, however, this form of activity began to taper off. Exploration went on, but took more the form of area

surveys in which the discovery of new sites was incidental to acquiring a knowledge of the area rather than an end in itself. There were the beginnings of studies concerned with the domestic houses and living patterns of the people, a new development in Maya research and one that was later to become extremely popular in American archaeology. Ceramic technology, the scientific study of the physical properties of pottery, was yet another new approach to archaeological problems. Almost certainly the most important aspect of the work of this period was the steadily increasing emphasis on "dirt" archaeology, the use of stratigraphic techniques of excavation to develop sequences of architecture, artifacts, and pottery.

There was also the important matter of extending the work to the Guatemala highlands. Two earlier fundamental misconceptions of the prehistory of Middle America were thus corrected. The idea that Classic Maya culture had its immediate beginnings in the highlands, whence it was carried to the previously uninhabited lowlands, was clearly untrue, as shown by the long sequence of pre-Classic, and surely indigenous, pottery underlying the Classic levels at Uaxactun. Secondly, the idea that the Maya were the first "civilizers" in Middle America, that they had given high culture to other peoples, was no longer tenable, for the widespread chronological equations established by the finds at Kaminaljuyu demonstrated that the Classic phases in other cultures were roughly coeval with that of the Maya, the earlier, underlying phases apparently being autochthonous. Lastly, it should be noted that Maya civilization was no longer looked upon as an isolated phenomenon but was regarded as one of the several high cultures of Middle America and as a part of the aboriginal civilization of the whole area. These were fundamental changes in thinking.

We are not sure that we have sufficiently stressed the size and the uniqueness of the

program of historical researches that the Institution had begun in 1930. This program, moreover, was not simply large; it was diverse, and it was experimental. Even omitting the studies outside Middle America, the only unifying force that applied to all segments of the work was the common area of operation. Unquestionably, some of the environmental studies had little regard for whether man had ever inhabited those parts or not. Some of the studies concerned with man had little interest as to his earlier, or perchance later, activities than those upon which the work focused. This is not to say that all studies were not providing knowledge pertinent to the understanding of man's career in Middle America. Virtually without exception they were. The problem was how to pull all this knowledge together, how to synthesize the results of the work, how to shape the great accumulation of knowledge, or potential knowledge, to the long-range purpose of the program—the story of man through the ages in Middle America. The more distant purpose, the effect and usefulness of that story in the over-all study of man and his works, was yet another matter.

It is clear that Kidder was well aware of the magnitude of these problems. In a number of his annual reports he points out the difficulty of coordinating such widespread researches, the problem of correlating and interpreting the results, the experimental nature of the program, and the need for an arbitrary time limit against the initiation of new work and for the completion of old. In his 1939 report, following a review of the first ten years of operation of the Division, he restates much of his earlier thinking on these matters and suggests a termination of researches and synthesizing of results over the following decade (*Year Book* 38, pp. 234–240). Before this report appeared in print war in Europe had begun, and two years later this country was actively involved.

It is hardly necessary to point out the disruption of research and plans for re-

search occasioned by the war. Quite aside from war, however, there were, and had for some time been, other forces at work that gravely affected the Division's operations. The broad program of allied researches envisioned by the President in 1923 and put into practice in 1930 had been predicated upon the expenditure of large sums of money. As the years went by and the costs of all research increased, it became progressively more difficult to finance so large an undertaking. Even at the peak of expenditures appropriations for the Division's work were approximately twenty per cent below the figure originally contemplated, and during the decade they averaged some twenty-five per cent below it. With the outbreak of war financial matters gave way to more pressing problems, but it became clear that in the future there would have to be a reappraisal of the activities of the Institution in general, and more specifically, from our point of view, of whether the broad program of historical researches involved a larger proportion of its resources than the Institution cared to allocate to such work.

At the end of hostilities the members of the staff of the Division, along with millions of other people, found themselves in a very different world from that of a few years earlier. The difference that concerns us here was a new climate in which to carry on their work. In his annual report for 1946 (*Year Book* 45, pp. 195-196), we find Kidder discussing the war's disruption of plans for an orderly termination, in the late 1940's, of the Division's program of researches, the need for reducing the extent of the Division's activities in the future, and the desirability of promptly formulating recommendations to the Institution for a new program of work to follow the termination of the old. The first of these problems can best be taken up in describing the research activities of the Division during the war and postwar years. In regard to the second

problem, it can simply be stated that by the end of 1950 all activities of the Division not directly related to archaeology had been concluded. Lastly, after a series of staff meetings, a new program of research, which will be described later, was presented to the President in 1947 and was approved in 1950.

Some research was, of course, carried on during the war. The work in social anthropology continued, although on a reduced scale. Hieroglyphic research went on without severe interruption. A project concerned with the architecture of the Maya, started just before the war, was brought to fruition in one of the Division's finest publications (*Publ.* 558). In the matter of archaeological field work, the winter of 1942 was active, mainly because a number of parties were already in the field, or on the point of departure, at the time of our entrance into the war. One such project, moreover, developed information of considerable importance to our understanding of Maya history. In his ceramic survey of Yucatan the late G. W. Brainerd found in a number of sites in the northern part of the peninsula pre-Classic, or Formative, period pottery. It will be remembered that a few years earlier the change in thought about the correlation of the Maya and Christian calendars had tended to push back in time the beginnings of civilization in northern Yucatan, but no one had thought very seriously of such beginnings as being earlier than the Classic period. With Brainerd's discovery it became evident that the northern culture was just as deeply rooted as that of the south, and equally indigenous. After the 1942 field season almost nothing was done except for some explorations in 1944. The preparation of manuscripts and publication of results of research, of course, continued through the war period.

Turning to the postwar years, it will readily be understood that the general process of contraction of the activities of the Division and the necessity of terminat-

ing in the space of five years a large and badly disrupted plan of researches left no place for a coordinated program of field work. Primary stress was upon the completion of earlier projects and upon the publication of the results of past research. How well this was accomplished is indicated by the appearance, from 1946 to 1950, of no less than twelve volumes in the Institution's monograph series, with eight more, all based on earlier work, to appear over the following five years. There were, moreover, other publications not under the Institution's imprint. Such field work as went on was mostly in the Guatemala highlands. It consisted mainly of archaeological survey and limited excavations. Somewhat more intensive digging, to a considerable extent unplanned in that it frequently originated from chance finds brought about by industrial and civic excavations, was carried on at the ruins of Kaminaljuyu on the outskirts of Guatemala City. The results of this work were extraordinarily profitable in that there was developed and defined a long sequence of Formative cultures extending considerably further back in time than had previously been known. An understanding of the relatively high development of Formative culture, moreover, did much to explain the apparently abrupt flowering of culture in succeeding Classic times. Another field project of major importance, also brought about by a chance find, was the recording and study of the now famous wall paintings at the lowland Maya site of Bonampak (Publ. 602).

It was mentioned earlier that hieroglyphic research continued relatively uninterrupted during the war and postwar years. This work was in part terminated in 1948 by the death of S. G. Morley, founder of the Institution's researches in Middle American archaeology, for thirty-five years a member of the staff, and a lifelong student of Maya hieroglyphic writing. There nevertheless appeared in 1950 the Institution's third great work on

Maya epigraphy (Publ. 589), this one by J. E. S. Thompson. Last, we should mention a project begun toward the end of the war that introduced a new method in the study of Maya sculpture. There is every chance that this work (Publ. 593) will stand as a milestone in the study of aboriginal Middle American art.

Certain activities of the Institution during this period, although not of a research nature, should be recorded. These included the assistance given to the government of Guatemala in establishing a museum of archaeology and ethnology and in the organization of a national institute of anthropology and history. Such activities, carried out on an unofficial level, constitute international cooperation of the best sort. Indeed, the entire history of the Institution's work in Middle America has been one of friendly dealings with governmental agencies and sincere cooperation with foreign colleagues. Surely not the least of the Institution's accomplishments has been the stimulus provided to the study of anthropology and archaeology in the countries where it has worked.

In reviewing this period of approximately a decade that included the war and postwar years, certainly the outstanding factors affecting the work of the Division were the disruption of research caused by the war and the change in thought by the Institution as to the part historical research should play in its over-all program of activities. In the first place, the long-considered and carefully laid plans that called for an orderly termination of the many and diverse studies under the historical program, to be followed by summation and synthesis of results, upon which were to be built recommendations for future work, were badly thrown awry by the war. Fortunately, there was time to bring the great majority of projects, with a few outstanding exceptions, to the point of publishing definitive results, but there was no place for summation and synthesis of the results of the whole, wide program

of studies. Second, the rapid retrenchment during the closing years of this period left the Division engaged almost solely in archaeology, with ceramic technology and a tenuous connection to aboriginal documentary history as the only collateral researches. This was, of course, a vast change from the large and expanding program of the 1930's.

In view of the difficulties that had to be overcome during these years, the results of the work were considerable. Of outstanding importance was the knowledge acquired of the Formative cultures of the Guatemala highlands and of Yucatan, and the light it threw upon the origins and development of Classic civilizations. Hieroglyphic studies had been measurably advanced, and a new approach to the study of pre-Columbian art had been created. Last, a large body of factual and definitive material had appeared in a long series of monographs and other published reports arising from the work of the Division.

Late in 1950 A. V. Kidder retired and the writer was appointed Director of the Department of Archaeology, the successor organization to the Division of Historical Research. The suggested program of operations, already mentioned, that had been presented to the President in 1947, had by this time been approved; it is presented in some detail in the annual report for 1951 (Year Book 50, pp. 221-224). The program was designed to be compact and to reach the stage of drawing conclusions in a predictable number of years. It was, of course, based primarily on archaeology but with considerable reliance on the results of previously performed historical research. The locus of the work was the Yucatan peninsula, and the period under consideration was the approximately five centuries preceding the Spanish conquest. The focal point of field operations was the last important center of aboriginal Maya civilization, the ruins of Mayapan. Subsidiary operations were archaeological sur-

veys and exploration in outlying areas thought to be important in the period under study, and an examination of certain known centers of Maya rule after the fall of Mayapan and during the final hundred years before the Spanish completed the conquest of Yucatan. The essence of the program, aside from more usual archaeological objectives, was an experiment in linking the results of archaeological research with the knowledge derived from aboriginal and early Spanish written records in the effort to discover how much of the intellectual, or at least nonmaterial, content of a bygone civilization could be recaptured.

It will be seen from the above that the earlier methods of intensive excavation at one large site, area surveys, and exploration were employed. At Mayapan, after the mapping of the ruins, particular emphasis was given to the study of the secular aspects of the culture, the dwellings of commoner and of noble, and the arrangement of houses within, and on the outskirts of, the city. Comparative work on a smaller scale was carried on elsewhere in the peninsula. Archaeological surveys, particularly stressing ceramics, were conducted in the little-known areas of Tabasco and coastal Quintana Roo. Exploration on a modest scale occurred in Yucatan, Quintana Roo, and along the west coast of the peninsula. Stratigraphic techniques of excavation, now firmly established in Middle American archaeology, were of course used extensively. In recognition of the friendly cooperation at all times offered by the Mexican government, the Institution voluntarily undertook a limited program of restoration of building remains, a project desired by that government.

Outside of archaeological field work documentary research continued, culminating in R. L. Roys' *Political Geography of the Yucatan Maya* (Publ. 613). In his hieroglyphic researches during this period Thompson turned to the gigantic task of

compiling a Maya hieroglyphic dictionary, a tool that should be of immeasurable value to future work in this field. Work in ceramic technology went on, resulting in the first definitive presentation of this subject from the point of view of usefulness to the archaeologist (Publ. 609). Other researches correlative to the central archaeological program were studies in physical anthropology dealing with the human skeletal remains from Mayapan, the identification and study of animal skeletal remains from that site, and the analysis of metal objects. The technique of dating archaeological remains by the newly developed radiocarbon method was also relied upon.

As this program of archaeological and related researches developed, the Institution made the decision to withdraw from the field of archaeology. Fortunately, the program had been shaped to reach the stage of drawing conclusions within approximately five years. Virtually all field work terminated in 1955, and the staff turned to the task of presenting the results of the work for publication.

The writer is hesitant in attempting to characterize the period from 1951 to the present. Not only has he been too closely identified with the work, but he lacks the perspective of time. Certainly one contrast to the preceding twenty years was the contraction in the size of the program and the restricting of the work to relatively clear-cut limits in time and space. Possibly the most important directing influences were the effort to link recorded history, both aboriginal and Spanish, with the results of archaeological field work, and the considerable attention devoted to the secular aspects of the life of the ancient Maya. Operating procedures did not vary in any marked degree from the preceding period, and the use of intensive excavation at one site was the revival of an earlier approach. A new technique, not peculiar to the work of the Institution but employed generally in American archaeology

at this time, was radiocarbon dating. Interestingly enough, because of certain radiocarbon dates there has been a marked trend among Middle American archaeologists to return to the previously little-favored Spinden correlation of the Maya and Christian calendars, a shift that affects our reconstructions not only of Maya history but of aboriginal Middle American history in general. Beyond this the writer does not feel that he should go in characterizing the work of the period.

He is even more hesitant about summarizing the results of the recent program. Some eight or nine manuscripts dealing with these results are at present in various stages of completion. The final story must rest on the information they present. In the meantime it seems possible to point to some failures, some successes. The effort to learn something of the last hundred years of aboriginal history in Yucatan, the time from the fall of Mayapan to the final conquest by the Spanish, was almost fruitless. In the matter of linking historical records with the information derived from archaeological field work, and the effort to develop some knowledge of the nonmaterial aspects of Maya civilization, it appears that we may not have gone as far as we had hoped but that our progress was not inconsiderable. In the annual reports of the period we are reviewing and in this Department's series of *Current Reports* will be found a number of references to the archaeological confirmation of previously unproved historical statements and to archaeological evidence concerning the social and religious aspects of Maya civilization. Chronological and cultural information of the sort normally to be expected from archaeology was of course produced. The time of greatness of Mayapan was well established, the character of its culture was delineated, and the relative chronology of, and the cultural ties, or lack of ties, between, central Yucatan, the east coast of the peninsula, and western Campeche and Tabasco were

outlined. Last, but not least, it was shown that the great break in cultural tradition, the precipitous step downward to degeneracy, occurred not with the rise to power, under foreign domination, of the great city of Chichen Itza, but at the end of that

city's time of greatness and with the rise of Mayapan. In broad outline these appear to be some of the results of the work of the past seven or eight years. Amplification must await the definitive reports now in preparation.

THE PRESENT

The work of the present year has, of course, been directed toward the termination of the activities of this Department. The major effort has gone into the preparation of reports presenting the results of our researches. At the end of the year, four such reports were finished, one was nearing completion, two others were well advanced, two were yet to be written. Quite naturally, no new field operations have been undertaken. Several members of the staff did, however, travel outside the country to complete their studies of archaeological collections.

R. E. Smith was in Yucatan approximately six months analyzing the large accumulations of ceramic remains from Mayapan and other sites in the peninsula. This phase of the work is now finished. A statement covering his recent activities appears in a subsequent section of this report. Smith will continue with the Institution for another year in order to prepare a monograph dealing with the pottery of Mayapan.

Aside from her primary task of preparing reports on the architecture and artifacts of Mayapan, Proskouriakoff spent two weeks in Yucatan in a final check of our collection of artifacts. She then continued to Guatemala, where she gave an additional two weeks to the examination of materials in the national museum that will be of value in her forthcoming studies. A report on this work will be found below. She will continue her studies, with the Institution, on the evolution of aboriginal art forms in Middle America.

Shepard, who has divided her time for the past year between the U. S. Geological Survey and this Department, has con-

tinued her researches in ceramic technology and in the composition of pigments from wall paintings and pottery decoration recovered from the ruins of Mayapan. During the winter she was able to spend three months in Yucatan and Guatemala, assisting R. E. Smith with his analysis of Yucatan pottery and rounding out some earlier research on pottery from Guatemala. Her report on this work will be found below. Shepard has recently been appointed geologist in the U. S. Geological Survey. She will continue for the present, however, to give some time to her ceramic technological researches for the Institution.

A. L. Smith has given the greater part of his time to a study of the residential structures and settlement patterns of Mayapan and its environs. In this work he is using comparative data from a number of other important sites in Yucatan. An interesting finding from these studies is the apparent similarity of the ancient groups of buildings, each group within its own walled enclosure, to the walled family lots in modern Indian villages in Yucatan. Some information about the function of structures is also emerging. In anticipation of his future work with the Peabody Museum of Harvard University, Smith spent approximately three months during the past winter in Guatemala, where he participated with Dr. Gordon R. Willey, of Harvard University, in a survey of ruins where the Peabody Museum is considering future operations. Smith left the Institution to take up his new work on July 1, 1958.

Thompson's principal activity during the past year has been the continuation of his

catalogue of Maya hieroglyphs. The work of listing all examples of every glyph, other than those of a strictly chronological nature, was concluded some time ago, and for some months past he has been engaged in transcribing this material into a catalogue which records every known combination of main element with affixes and the location on monument or codex of each combination. This is a long and tedious task, for often a main sign can be identified, whereas the smaller affixes, more easily affected by weathering, are difficult to make out. Up to the end of April Thompson had catalogued, with some commentary, 95 main elements, all of the geometric category, representing 4940 occurrences.

Thompson has also given some attention to locating by means of local glyphs the sites at which certain artifacts, or at least the glyphs on the artifacts, were carved or incised. This study should throw light on local styles and trade routes. At a later date he hopes to publish a short paper on the subject. On his retirement from the Institution in the near future, he will continue his researches in England, where he has taken up residence.

During the past year Shook has remained on leave of absence in order to take charge of the work of the University Museum, University of Pennsylvania, at the ruins of Tikal in Guatemala. With his retirement from the Institution on July 1, 1958, Shook will continue his work with the University Museum.

Shortly after the close of the year under review, Lillian Lawrence, Administrative Secretary of the Department for the past eight years, will leave the Institution. The Director and all members of the staff are deeply indebted to Miss Lawrence for her unflinching assistance, efficiently and cheerfully performed, in administrative and many other matters. She will take up new duties in business.

Several administrative happenings of

the last twelve months should be recorded. In November the Director went to Yucatan to effect the turning over of a considerable portion of our archaeological collections to the Instituto Nacional de Antropología e Historia of Mexico and to arrange for the closing on January 1, 1958, of the Department's offices and laboratory in Merida. The remainder of the collections was delivered to the Instituto during the past winter and spring. A detailed and carefully arranged documentation accompanied these materials.

The disposing of the equipment and scientific records of the Department, a process that has gone on for several years, was brought to a conclusion by the end of the year under review, with the exception of such materials at our laboratory of ceramic technology at Boulder, Colorado, where Shepard will continue her researches for the present. It has been the policy of the Institution that equipment should be disposed of so as to continue in the uses for which it was originally acquired. Following this policy, gifts have been made to the University Museum of the University of Pennsylvania, to the Middle American Research Institute of Tulane University, and to the R. S. Peabody Foundation for Archaeology at Andover, Massachusetts. The major part of our field and office equipment in Yucatan has been presented to the Instituto Nacional de Antropología e Historia of Mexico. Scientific equipment, office equipment in Cambridge, and virtually all our scientific records have been given to the Peabody Museum of Harvard University, an organization with which this Department has closely co-operated for many years.

In the autumn of 1957 J. Eric S. Thompson was elected a member of the Faculty Board of Archaeology and Anthropology, Cambridge University. Tatiana Proskouriakoff has recently been appointed Research Fellow in Maya Art, Peabody Museum, Harvard University.

CERAMIC STUDIES IN YUCATAN

R. E. Smith

During the past season Smith's work embraced four main projects: a recheck of the ceramic material from Kabah, Uxmal, and Chichen Itza, studied last season, and an integration of the results of the technological analysis of this material made by Shepard in 1958; completion of the recording of data on the pottery of Mayapan; the selection of Mayapan pottery types as material for sherd libraries for various institutions in the United States and Mexico; and the organization in cases of all the ceramic material to be turned over to the Instituto Nacional de Antropología e Historia of Mexico.

Since he had previously studied the material from Kabah, Uxmal, and Chichen Itza, it was a simple matter to introduce and to coordinate the technological data found by Shepard. This new information added greatly to the understanding and description of the wares and types from these sites. For example, ash temper formed 66.5 per cent of all tempers used in the sherds examined from the Toltec Phase at Chichen Itza, as compared with 19.4 and 33.7 per cent from the latest phase at Uxmal and Kabah, respectively. This predominant use of ash temper suggested a considerable deposit of that material at or near Chichen Itza, and with this in mind Shepard checked the area briefly during a two-day visit to the site but found no evidence of ash. This fact does not, however, eliminate Chichen Itza, or some place near by, as the source of the wind-blown ash used as temper, since Shepard had insufficient time to make a thorough search of the site and did not investigate the surrounding areas. Ash of wind-blown origin was used as temper, though to a lesser degree, in the Puuc area, at least on pottery pertaining to the latest phase at Uxmal and Kabah. It is possible, then, that ash was employed somewhat sparingly by potters who had the material at hand during the Classic Period, and that the use was greatly increased during the Early Mexican

Period in Yucatan, owing to the advent and influence of the Toltecs who dominated Chichen Itza during that period. These Toltecs, coming from a volcanic region in Mexico, may well have been prejudiced in favor of ash temper.

Prior to this season Smith had made a careful study of the pottery associated with the earliest and latest deposits at Mayapan. This year his efforts were concentrated on the pottery found midway between the earliest and the latest levels. The middle levels at Mayapan contained a very substantial part of the total sherd material examined, and for that reason, as well as others, they are of great importance. The result of this work was that, in Smith's opinion, there is no identifiable middle ceramic phase. The early types extend into the middle levels, gradually diminishing, and the late types occur in small quantities, gradually increasing until in the surface levels they predominate.

The selection of Mayapan pottery to be sent to various institutions in the United States and Mexico involved seven types: Mayapan Unslipped, Mayapan Cream, Mayapan Black-on-cream, Mayapan Red, Mayapan Red-on-buff, Mayapan Red-and-black-on-buff or -on-orange, and V Fine Orange. Examples of all of them, including most of the shapes, decorative techniques, and styles of design, were chosen for shipment to each institution.

The organization of the ceramic material in the Institution's laboratory in Yucatan was on a typological basis. This material, together with all other artifacts, will shortly be transferred by the Instituto Nacional to the recently formed Instituto de Antropología e Historia de Yucatan in Merida. There are collections from 87 sites: Quintana Roo, 29; Yucatan, 21; Tabasco, 18; Campeche, 16; Chiapas, 2; Veracruz, 1. Of these collections the largest and most thoroughly studied are: from Yucatan—Mayapan, in which the types are arranged according to early, middle,

and late levels, Chichen Itza, Uxmal, Kabah, and Mani; from Quintana Roo—Tulum, Ichpaatun, and Tancah; from Tabasco—Jonuta, Juarez, Tamulte, and Hui-mango; from Campeche—Atasta, Santa Rosa Xtampak, Xpuhil, and Dzibilnocac (Iturbide); from Chiapas—Tecalpan. A report on the pottery from Atasta, Hui-mango, Jonuta, Juarez, Tamulte, and Tecolpan has been published by H. Berlin. W. T. Sanders has prepared, and will shortly publish, a study of the collections from Calderitas, Ichpaatun, Tancah, and

Tulum. A report by R. E. Smith on the early post-Classic Period or Toltec Phase pottery at Chichen Itza and that of the latest ceramic period (Late Classic and early post-Classic transitional) at Uxmal and Kabah is nearing completion, and a full report by the same author on the pottery of Mayapan is under preparation. Last, a general study by the late G. W. Brainerd of the pottery of Yucatan, which will include most of the Yucatecan sites in the collection, is expected to appear in the near future.

ART AND ARTIFACTS

Tatiana Proskouriakoff

To check on some questions that had arisen in the course of preparing a report on the artifacts of Mayapan, Proskouriakoff spent two weeks in November in Merida. At the same time, 50 new specimens from Chichen Itza and the Puuc region were catalogued, and the Merida copy of the catalogue was checked and arranged for consignment to the Instituto Nacional de Antropología e Historia of Mexico.

The following two weeks were devoted to the examination of ceramic collections in the Museo Nacional de Arqueología y Etnología in Guatemala City, with the object of appraising their relevance to a projected study of the evolution of art forms in Middle America. The collections are admirably organized and include a large body of material representing sequent stages of development in highland Guatemala before the Classic Period of Maya art. The sequences have been worked out by Kidder and Shook in their collaborative work on the archaeology of the region, but only a very small part of the material has been published. From the earliest stages represented, the pottery is

richly decorated and continuities in motifs and in formal treatment can be traced over considerable spans of time. Although it is not yet clear on what basis more or less independent traditions of ornament can be distinguished, it was observed that a small number of early wares employ designs that are clearly related to basic motifs of Classic Maya art. It is therefore possible that an exposition of continuities in developing traditions will draw definite limits to the degree and nature of artistic influences that can be ascribed to outside sources, and thus may restrict the field of speculation concerning similarities found in widely separated styles such, for example, as have been adduced in support of the hypothesis of trans-Pacific contacts.

In addition to opening possibilities of tracing back the history of Classic motifs, the pre-Classic collections, because of the restricted range of the designs and their relative simplicity, present a body of material peculiarly adapted for testing various methods of describing and representing the changes that take place in a complex of decorative styles as the culture passes through successive phases of development.

CERAMIC TECHNOLOGY

Anna O. Shepard

During the current year Shepard, working on a half-time basis, has reviewed technological data on Yucatan and lowland

Maya pottery in order to prepare this material for publication while transferring to geology as her major field of endeavor.

Much of our analytical work on the ceramic technology of these areas was undertaken before the archaeologist's stylistic studies were completed. Our principal need, therefore, was to round out our data and establish a broader base for interpretations. This necessitated study of collections in Yucatan and Guatemala.

Shepard spent twelve weeks in the field seeking correlation between stylistic and technical features. From January 10 to February 7 she worked in Merida on R. E. Smith's collections from test cuts at Uxmal, Kabah, and Chichen Itza. A clear-cut distribution pattern and definite relations between paste composition, ware, and vessel shape emerged from this review. Some wares, such as the fine-textured, calcite-tempered Thin Slate and the coarse, sherd-tempered Holactun Black-on-cream, are uniform in composition, whereas the more generalized ware Puuc Slate is heterogeneous, including four paste classes: calcite-, volcanic ash-, and sherd-tempered pastes, and lumpy untempered paste. A number of varieties of calcite temper further diversify this ware. A similar contrast is found in the stylized vessel shapes. Calcite-tempered or volcanic ash-tempered paste is strongly preponderant in some forms, whereas others include a significant representation of the main paste classes. The degree of paste uniformity also differs by site. The two classic Puuc sites, Uxmal and Kabah, have a heterogeneous paste representation; in contrast, the Chichen Itza sample, classed as Toltec by Smith, is exceptionally uniform, the various slipped types running almost exclusively to volcanic ash temper and the unslipped types to calcite temper, with high correlation between classes of calcite and vessel form. We have, then, some good correlations between paste, ware, and shape, some centers showing well established techniques, others exhibiting technical diversity. These occurrences are most simply explained by the hypothesis that pottery-making communities drew on diverse local resources and that there was a lively trade among

them, some communities being much more dependent on exchange than others. That is, standardized wares and types may be considered products of pottery-making communities having well established techniques; classes that are variable in composition suggest that style was more widely established than technique; uniformity within a site may reflect self-sufficiency in pottery production; diversity may indicate a community depending in large measure on trade for its pottery. These explanations are without doubt glaring oversimplifications. There are other factors that must be weighed, especially exchange of raw materials, potters of a community practicing a number of different techniques or passing through a period of experimentation, and different degrees of standardization in different centers. These explanations should all be tested as working hypotheses in future explorations, and this season's results hold promise that definitive answers can be obtained.

Several collections from other sites were examined for comparative purposes. The most interesting was a small sample of Tulum Redware. Its calcite-tempered paste was distinguished from this class of paste in the Puuc sites, Chichen Itza, and Mayapan by the presence of quartz grains. This diagnostic served to identify as trade ware some of the sherds from a small Mayapan lot selected by Smith on the basis of surface features as possibly Tulum Red.

While in Merida, Shepard examined a sample of the principal wares and some unusual pieces from the National Geographic-Tulane University excavations at Dzibilchaltun. It was gratifying to find sufficient interest in the results to give promise that this line of investigation will be followed independently.

Two pottery-making villages, Lerma and Tepakan, were visited to observe firing methods, and a record of firing temperature was obtained from the work of a Ticul potter at Hacienda Uxmal.

An attempt to obtain information on the source of the pigment Maya blue and

to get a sample of it from the village of Tekax, where it is rumored to occur, was unsuccessful. During the year, however, additional analytical data on this pigment were secured. A record of reflectance spectra was made through the courtesy of Dr. R. O. Fehr, of the General Electric Company. The tristimulus values calculated from such curves serve to identify pigments, but no published values corresponding to those obtained for Maya blue have been found. Shepard has considered the possibility that this pigment is an organic dye held by a clay with an expanding lattice. It was hoped that a clue to the presence or absence of an organic constituent might be obtained from an infrared transmission spectrum. Dr. Hans B. Gottlieb and Mr. Bert Weberg, of the Chemistry Department of the University of Colorado, kindly ran spectra for Maya blue and hematite as a control. The Maya blue curve corresponded to that of a rare clay mineral, but one peak, which might represent an organic constituent, was too faint to be definitive. A dye, if present, would make up a small percentage of the material, and we are still handicapped by lack of an adequate sample.

In Guatemala from February 7 to March 30 Shepard's principal project was a re-examination of the Uaxactun collection. She had established the sequence of paste classes in previous seasons. It is surprising that sherd, a superior tempering material that predominated in Mamom ware, was replaced by the inferior material, calcite, for unslipped Chicanel ware, and in the following phase, when ceramic progress was expressed by the advent of polychrome ware, sherd temper was abandoned almost completely. It was in the Tzakol phase that volcanic ash was first used extensively, and the great bulk of early and middle Tepeu fine ware was tempered with this material. Unlike the volcanic ash of Yucatecan ware, this shows considerable diversity in texture. Especially noteworthy is the associated mineral matter, which is high enough in one variety

to class it as a crystal-vitric ash. It seems improbable from the coarseness of the ash that it was air borne this distance. The sources of these ash tempers of the Peten are as yet undetermined. Geologists from a number of oil companies having concessions in the Peten were interviewed but could offer no clues.

Correlations between paste composition and defined style were not high, suggesting that Uaxactun potters were using a variety of pastes indiscriminately or that Uaxactun was obtaining pottery from an area in which a single stylistic tradition held but technique and resources differed, or that our sherds were not giving adequate stylistic criteria. When attention was directed to finishing techniques, color classification previously established was found inadequate. It had not been related to basic finishing method and was sometimes misleading because the colors of distinct slips often intergrade and a given slip may exhibit wide color variations depending on postdiscard environment. The fullest evidence was obtained from the painted wares of the Classic Period. There are two distinct classes of slip: one is whitish to buff, earthy, nearly lusterless, and was applied as a relatively thick coat; the other, which ranges from yellow through orange to red, has a high lacquer-like luster and is very thin. Frequently, both these materials are used on the same vessel, the lustrous one always forming the final coat. Each, however, was also used alone, and a fourth technique consisted in smoothing and polishing without the application of a contrasting coat. Microscopic examination has further broken down the light slip into calcareous, non-calcareous, and ash-tempered varieties. The composition of the lacquer-like coat remains to be defined by laboratory analysis. These four basic finishing techniques are primary diagnostics and in a number of instances showed close correlation with style and paste.

Two outstanding features of Classic Peten decoration are the range of colors

obtained by mixing pigments and specialized resist techniques. Pigment mixtures include gray from manganese and calcite, a lavender pink from hematite red and calcite, and brown from manganese and hematite. The constancy of color of the brown, which was used to represent body color in figure painting, indicates the maintenance of similar proportions of the two pigments. A striking feature of the resist decoration is the employment of hematite red paint in contrast to the carbon black which is the standard pigment for this type of decoration in both North and South America. Evidence was also found for two successive applications of the resist material and the use of two colors in the resist process itself, which reflect an unusual elaboration of this specialized technique.

Other collections examined for comparative purposes included that from Piedras Negras, which presents marked uniformity in paste composition in contrast to Uaxactun. Shepard also spent two days at Tikal examining a sample of the pottery and reviewing the problems of paste analysis for Miss Broman, of the University Museum. A small collection of special interest was that made by Dr. Willey, of the Peabody Museum, at Altar de Sacrificios, a site long suspected of being a trade center. A number of pastes were defined, the sources of which cannot at present be located because of our limited knowledge of distributions in this area.

Short trips were made to Zaculeu, with a stop at Quetzaltenango to examine the Robles collection from Finca Paraiso, and to Cahyup near Rabinal. The latter trip

indicated the feasibility of attacking the problem of trade and centers of pottery manufacture by defining petrographic provinces. Cahyup is in the Precambrian, well beyond the region of recent volcanics. The pottery reflects this location. Schist or quartzite was present in the three classes of paste collected. The location of petrographic provinces would be guided by available geologic maps and, in collecting, due attention would be given to the smaller sites which may have relatively less trade ware than the large prominent ones. This approach seems worth pursuing.

During August 1957 Shepard attended a six-day National Clay Conference in Berkeley, returning by way of Tucson to visit the Peabody Museum laboratory there and to attend the Pecos Conference in Globe. The Awatovi pottery which Mr. Watson Smith and Mr. Robert Burgh are analyzing in Tucson presents different technological problems from those generally encountered. The most significant evidence is not of trade but rather of the course of local ceramic development, and the most important changes are not in tempering material but in the class of clay and in firing method. Our test specimens and notes have been turned over to Mr. Watson Smith, and further discussions with him are planned.

In bringing the Ceramic Technology Project to a close Shepard will concentrate on the completion of reports. She will also be glad to aid, in whatever way is feasible, students interested in taking up any aspect of technological analysis.

PUBLICATIONS

H. E. D. Pollock

The manuscripts of three reports in a forthcoming monograph have been received and are ready for editing. These are tentatively titled: *Literary Sources for the History of Mayapan*, by Ralph L. Roys, *Civic and Religious Structures and Monu-*

ments of Mayapan, by Tatiana Proskouriakoff, and *Artifacts of Mayapan*, by Tatiana Proskouriakoff.

The manuscript of a paper in volume XII of Contributions to American Anthropology and History has also been re-

ceived: *Ceramics and Settlement Patterns in Quintana Roo, Mexico*, by William T. Sanders.

The fifth volume of Notes on Middle American Archaeology and Ethnology was completed during the past year with the addition of one paper: *The Márquez Collection of X Fine Orange and Fine Orange Polychrome Vessels* (no. 131), by Robert E. Smith. With the issue in May of the table of contents and index for this volume, this series of publications by the Department came to an end.

Volume II of Current Reports was concluded during the year with two more reports and a separate map: *Deities Portrayed on Censers at Mayapan* (no. 40), by J. Eric S. Thompson; *Notes on Vertebrate Animal Remains from Mayapan* (no. 41), by H. E. D. Pollock and Clayton E. Ray; *Topographic Map of the Ruins of Mayapan, Yucatan, Mexico* (revised edition). This series of publications was also terminated in May with the issue of the table of contents of volume II and an index for volumes I and II.

BIBLIOGRAPHY

July 1, 1957—June 30, 1958

- Pollock, H. E. D., and C. E. Ray. Notes on vertebrate animal remains from Mayapan. Carnegie Inst. Wash., Dept. Archaeol., Current Reports, no. 41 (1957).
- Proskouriakoff, Tatiana. Notes and news: Middle America. *Am. Antiquity*, 23, 218–220 (1957); 460–462 (1958).
- Proskouriakoff, Tatiana. Review of Pál Kelemen, Medieval American art, masterpieces of the New World before Columbus. *Am. Antiquity*, 23, 190–191 (1957).
- Ray, Clayton E. See Pollock, H. E. D.
- Shook, Edwin M. Lugares arqueológicos del altiplano meridional central de Guatemala. In *Arqueología Guatemalteca*, pp. 65–130. Inst. de Antropología e Historia de Guatemala, Publ. 20 (1957).
- Shook, Edwin M. Estado actual de las investigaciones en el horizonte preclásico de Guatemala. Inst. de Antropología e Historia de Guatemala, *Antropología e Historia*, 9, no. 2, pp. 3–11 (1957).
- Shook, Edwin M. Una escultura Olmeca de Guatemala. Inst. de Antropología e Historia de Guatemala, *Antropología e Historia*, 9, no. 2, pp. 12–16 (1957).
- Shook, Edwin M. Una escultura Olmeca de Guatemala. *Fichero*, no. 2, pp. 20–22. Guatemala (1957).
- Shook, Edwin M. Tikal—1957 season. *Archaeology*, 10, 221–223 (1957).
- Shook, Edwin M. The Tikal project. Univ. Penn., *Univ. Mus. Bull.*, 21, no. 3, pp. 37–52 (1957).
- Smith, Robert E. The Márquez collection of X fine orange and fine orange polychrome vessels. Carnegie Inst. Wash., Dept. Archaeol., Notes on Middle Am. Archaeol. and Ethnol., no. 131 (1957).
- Thompson, J. Eric S. Grandeur et décadence de la civilization maya. Payot, Paris (1958).
- Thompson, J. Eric S. Maya: En kulturs blomstring och fall. Tidens Förlag, Stockholm (1958).
- Thompson, J. Eric S. Thomas Gage's travels in the New World. Edited with an introduction and notes by J. Eric S. Thompson. University of Oklahoma Press, Norman (1958).
- Thompson, J. Eric S. Tentativa de reconocimiento en el area Maya meridional. In *Arqueología Guatemalteca*, pp. 23–63. Inst. de Antropología e Historia de Guatemala, Publ. 20 (1957).
- Thompson, J. Eric S. Deities portrayed on censers at Mayapan. Carnegie Inst. Wash., Dept. Archaeol., Current Reports, no. 40 (1957).
- Thompson, J. Eric S. Symbols, glyphs, and divinatory almanacs for diseases in the Maya Dresden and Madrid codices. *Am. Antiquity*, 23, 297–308 (1958).

PERSONNEL

July 1, 1957—June 30, 1958

H. E. D. Pollock, *Director*
Lillian E. Lawrence, *Administrative Secretary*
Tatiana Proskouriakoff
Anna O. Shepard

Edwin M. Shook *
A. Ledyard Smith
Robert E. Smith
J. Eric S. Thompson

* Absent on leave.

BIBLIOGRAPHY July 1, 1957—June 30, 1958

PUBLICATIONS OF THE INSTITUTION

Year Book 56, 1956-1957. Octavo, xliii+425 pages, 14 plates, 131 figures. December 1957.

175. Volume xx. Isabelle Lange and S. E. Forbush. *Cosmic-Ray Results. Huancaayo, Peru, January 1946-December 1955; Cheltenham, Maryland, March 1936-December 1955; Christchurch, New Zealand, January 1947-December 1955; Godhavn, Greenland, January 1947-December 1950.* Quarto, v+224 pages, 431 tables. August 1957.

581. Second printing. Jens Clausen, David D. Keck, and William M. Hiesey. *Experimental Studies on the Nature of Species. III. Environmental Responses of Climatic Races of Achillea.* Octavo, iii+129 pages, 30 figures, 21 tables. June 1958.

607. Second printing with brief addenda. Richard B. Roberts, Philip H. Abelson, Dean B. Cowie, Ellis T. Bolton, and Roy J. Britten. *Studies of Biosynthesis in Escherichia coli.* Octavo, xiv+521 pages, 116 figures. September 1957.

609. Second printing. Anna O. Shepard. *Ceramics for the Archaeologist.* Octavo, xii+414 pages, 59 figures. August 1957.

610. Second printing. Paul W. Merrill. *Lines of the Chemical Elements in Astronomical Spectra.* Quarto, vii+167 pages, 2 figures, 36 diagrams. 1958.

611. *Contributions to Embryology*, volume xxxvi. Quarto, iii+210 pages, frontispiece, 59 plates, 52 text figures. November 15, 1957.

242. Osborne O. Heard. Methods used by C. H. Heuser in preparing and sectioning early embryos. Pages 1-18, 3 plates, 12 text figures.

243. Benjamin C. Moffett, Jr. The prenatal development of the human temporomandibular joint. Pages 19-28, 2 plates.

244. Chester H. Heuser and George W. Corner. Developmental horizons in human embryos. Description of age group x, 4 to 12 somites. Pages 29-39, 2 plates, 2 text figures.

245. E. Ide Smith. The early development of the trachea and esophagus in relation to atresia of the esophagus and

tracheoesophageal fistula. Pages 41-57, 4 plates, 11 text figures.

246. Perry W. Gilbert. The origin and development of the human extrinsic ocular muscles. Pages 59-78, 14 plates, 2 text figures.

247. Dorcas H. Padgett. The development of the cranial venous system in man, from the viewpoint of comparative anatomy. Pages 79-140, 6 plates, 18 text figures.

248. E. Carl Sensenig. The development of the occipital and cervical segments and their associated structures in human embryos. Pages 141-152, 4 plates.

249. George W. Bartelmez. The form and the functions of the uterine blood vessels in the rhesus monkey. Pages 153-182, 15 plates.

250. Ronan O'Rahilly, D. J. Gray, and Ernest Gardner. Chondrification in the hands and feet of staged human embryos. Pages 183-192, 1 plate.

251. Roy E. Crowder. The development of the adrenal gland in man, with special reference to origin and ultimate location of cell types and evidence in favor of the "cell migration" theory. Pages 193-210, 8 plates, 7 text figures.

614. Hermann Moser. *The Dynamics of Bacterial Populations Maintained in the Chemostat.* Octavo, iv+136 pages, 47 figures, 18 tables. February 1958.

615. Jens Clausen and William M. Hiesey. *Experimental Studies on the Nature of Species. IV. Genetic Structure of Ecological Races.* Octavo, vii+312 pages, 33 figures, 68 tables. May 1958.

PUBLICATIONS BY THE PRESIDENT OF THE INSTITUTION

Caryl P. Haskins

Review of *A History of Technology* (Library of Science, New York). *Library of Science Book News*, volume 4, no. 1, pages 4-6. September 1957.

Report of the President. *Carnegie Institution of Washington Year Book* 56, July 1, 1956-June 30, 1957, pages 1-33. Carnegie Institution of Washington, Washington, D. C. December 1957.

ADMINISTRATIVE REPORTS

REPORT OF THE EXECUTIVE COMMITTEE

To the Trustees of the Carnegie Institution of Washington:

Gentlemen: In accordance with the provisions of the By-Laws, the Executive Committee submits this report to the annual meeting of the Board of Trustees.

The estimate of expenditures for the fiscal year beginning July 1, 1958, has been reviewed by the Executive Committee.

One vacancy exists in the membership of the Board of Trustees.

The terms of the following Officers and Committee Members have expired during the past year:

Officers of Board of Trustees

Walter S. Gifford, Chairman
Barklie McKee Henry, Vice-Chairman
Robert Woods Bliss, Secretary

<i>Executive Committee</i>	<i>Finance Committee</i>	<i>Auditing Committee</i>
Henry S. Morgan	Walter S. Gifford	Alfred L. Loomis
Henning W. Prentis, Jr.	Alfred L. Loomis	Keith S. McHugh
Henry R. Shepley	Henry S. Morgan	Juan T. Trippe
 <i>Retirement Committee</i>	 <i>Nominating Committee</i>	
Barklie McKee Henry	Crawford H. Greenewalt	
Lindsay Bradford		

Barklie McKee Henry, Chairman of Executive Committee
Lindsay Bradford, Chairman of Finance Committee
Keith S. McHugh, Chairman of Auditing Committee
Lindsay Bradford, Chairman of Retirement Committee
Elihu Root, Jr., Chairman of Nominating Committee

Committee on Astronomy: Seeley G. Mudd, Chairman, Crawford H. Greenewalt, Elihu Root, Jr.

Committee on Terrestrial Sciences: Ernest O. Lawrence, Chairman, Barklie McKee Henry, Henning W. Prentis, Jr., Robert E. Wilson

Committee on Biological Sciences: Alfred L. Loomis, Chairman, Margaret Carnegie Miller, William I. Myers, Charles P. Taft

Committee on Archaeology: Henry R. Shepley, Chairman, James F. Bell, Robert Woods Bliss, Juan T. Trippe

BARKLIE MCKEE HENRY, *Chairman*

May 9, 1958

ACCOUNTANTS' REPORT

LYBRAND, ROSS BROS. & MONTGOMERY

ACCOUNTANTS AND AUDITORS

NEW YORK	DETROIT	BIRMINGHAM
PHILADELPHIA	CLEVELAND	DALLAS
CHICAGO	CINCINNATI	HOUSTON
BOSTON	ROCKFORD	TULSA
BALTIMORE	ST. LOUIS	SAN FRANCISCO
WASHINGTON	LOUISVILLE	LOS ANGELES
PITTSBURGH	HARTFORD	SEATTLE

COOPERS & LYBRAND

IN AREAS OF THE WORLD
OUTSIDE THE UNITED STATES

To the Auditing Committee of Carnegie Institution of Washington:

We have examined the statement of assets, liabilities and fund balances of Carnegie Institution of Washington as of June 30, 1958, and the related summary statement of changes in funds for the year then ended and the supporting exhibits and schedules, which have been prepared on the general basis of cash receipts and disbursements and accordingly do not reflect accrued income, accounts payable nor provision for depreciation. Our examination was made in accordance with generally accepted auditing standards, and accordingly included confirmation from the custodian of securities owned at June 30, 1958, and such tests of the accounting records and such other auditing procedures as we considered necessary in the circumstances.

In our opinion, the accompanying financial statements and supporting exhibits and schedules present fairly the assets, liabilities and fund balances of Carnegie Institution of Washington at June 30, 1958, and the changes in funds for the year then ended on a basis consistent with that of the preceding year.

LYBRAND, ROSS BROS. & MONTGOMERY

Baltimore, Maryland
September 9, 1958

STATEMENT A

ASSETS, LIABILITIES AND FUND BALANCES
JUNE 30, 1958 AND 1957

	June 30	
	1958	1957
ASSETS		
<u>Operating Funds:</u>		
Cash	\$535,096	\$331,367
Advances	6,177	15,406
Securities - Schedule 1 (See Note)	357,332	325,073
Prepaid insurance	33,789	35,789
	<u>\$932,394</u>	<u>\$707,635</u>
<u>Restricted Grants:</u>		
Cash	\$362,799	\$134,591
<u>Endowment, General Reserve, and Special Funds:</u>		
Cash awaiting investment	\$11,627	\$51,850
Securities - Schedule 1 (See Note)	55,568,043	55,306,013
	<u>\$55,579,670</u>	<u>\$55,357,863</u>
<u>Buildings, Land, and Equipment</u> (At Cost)	<u>\$5,537,150</u>	<u>\$5,375,541</u>
Total Assets	<u><u>\$62,412,013</u></u>	<u><u>\$61,575,630</u></u>
LIABILITIES AND FUNDS		
<u>Operating Funds:</u>		
Income taxes, etc., withheld	\$3,599	\$4,519
Operating Funds Balance - Exhibit 1	928,795	703,116
	<u>\$932,394</u>	<u>\$707,635</u>
<u>Restricted Grants</u> - Exhibit 2	<u>362,799</u>	<u>134,591</u>
<u>Endowment, General Reserve, and Special Funds</u> - Exhibit 3	<u>55,579,670</u>	<u>55,357,863</u>
<u>Buildings, Land, and Equipment Fund</u> - Exhibit 4	<u>5,537,150</u>	<u>5,375,541</u>
Total Liabilities and Funds	<u><u>\$62,412,013</u></u>	<u><u>\$61,575,630</u></u>

Note: Approximate market value of all securities at June 30, 1958 - \$74,351,890

STATEMENT B

SUMMARY STATEMENT OF CHANGES IN FUNDS
FOR THE YEAR ENDED JUNE 30, 1958

	Operating Funds (Exhibit 1)	Restricted Grants (Exhibit 2)	Endowment, General Reserve, and Special Funds (Exhibit 3)	Buildings, Land, and Equipment (Exhibit 4)	Total
Balance July 1, 1957	\$703,116	\$134,591	\$55,357,863	\$5,375,541	\$61,571,111
<u>Additions:</u>					
Investment income	\$1,043,495	\$1,562,080	\$2,605,575
Restricted grants	\$383,537	383,537
Dormitory	11,032	11,032
Sales of publications	8,288	8,288
Other income	2,500	11,206	13,706
Realized capital gain, net	47,112	47,112
Current year's expenditures capitalized	167,724	167,724
Prior years' expenditures cap- italized	9,178	9,178
By transfer:					
Budget appropriation - Jan- uary 1, 1958 to June 30, 1958	1,394,291	(1,394,291)
	<u>\$2,459,606</u>	<u>\$383,537</u>	<u>\$226,107</u>	<u>\$176,902</u>	<u>\$3,246,152</u>
<u>Deductions:</u>					
Expenditures	\$2,233,927	\$155,329	\$4,300	\$2,393,556
Disposition of land and equipment	\$15,293	15,293
	<u>\$2,233,927</u>	<u>\$155,329</u>	<u>\$4,300</u>	<u>\$15,293</u>	<u>\$2,408,849</u>
Net change during the year	<u>\$225,679</u>	<u>\$228,208</u>	<u>\$221,807</u>	<u>\$161,609</u>	<u>\$837,303</u>
Balance June 30, 1958	<u>\$928,795</u>	<u>\$362,799</u>	<u>\$55,579,670</u>	<u>\$5,537,150</u>	<u>\$62,408,414</u>

EXHIBIT 1

CHANGES IN OPERATING FUNDS FOR
THE YEAR ENDED JUNE 30, 1958

Balance July 1, 1957		\$703,116
----------------------------	--	-----------

Income - Statement B:

Investment Income	\$1,043,495	
Dormitory	11,032	
Sales of publications	8,288	
Other income	2,500	1,065,315
		<hr/>

<u>Transfer from General Reserve Fund - Budget Appropriation January 1,</u> 1958 to June 30, 1958 - Exhibit 3		1,394,291
--	--	-----------

Total available for expenditure		\$3,162,722
---------------------------------------	--	-------------

Expenditures:

Salaries.....	\$1,211,948
Equipment	165,224
Laboratory	174,000
Buildings - Fuel, Lights, etc.	81,359
Shop	11,427
Travel	15,657
Dormitory	11,190
Operating	101,733
Insurance Premiums	19,196
Financial Administration - Investment and Custody Fees	35,982
General publications	31,812
Other publication expense	9,696
Fellowships	65,692
Retirement Plan Contributions	155,599
Pensions, group insurance premiums, and social security taxes	88,154
Hospitalization Plan	22,422
Miscellaneous	32,836
	<hr/>

Total expenditures		2,233,927
--------------------------	--	-----------

Balance June 30, 1958		\$928,795
		<hr/>

EXHIBIT 2

CHANGES IN RESTRICTED GRANTS
FOR THE YEAR ENDED JUNE 30, 1958

	Unexpended Balance July 1, 1957	Grants Received	Expendi- tures	Unexpended Balance June 30, 1958
<u>Departmental Research Operations:</u>				
Department of Genetics:				
American Cancer Society No. EG-21	\$5,010	\$5,010
American Cancer Society No. E-55	\$7,600	5,792	\$1,808
American Cancer Society No. E-10	5,500	4,057	1,443
U. S. Public Health Service No. RG-149C	2,995	9,637	10,199	2,433
U. S. Public Health Service No. C-2158C	3,108	12,000	13,104	2,004
Department of Terrestrial Magnetism:				
National Science Foundation No. 1196-1	4,760	4,200	560
National Science Foundation No. 2699	4,727	3,680	1,047
National Science Foundation No. Y/3.16/169 .	19,708	18,541	1,167
National Science Foundation No. Y/2.34/291	12,000	12,000
National Science Foundation No. Y/11.11/170	21,200	6,000	27,200
National Science Foundation No. 3549	9,000	9,000
National Science Foundation No. 5410	255,000	255,000
Department of Embryology:				
Population Council, Inc.	2,511	8,500	10,532	479
Mount Wilson Observatory:				
Anonymous	3,500	2,000	2,500	3,000
<u>Publications:</u>				
Blake, Marion E.	3,300	3,300
Guggenheim Memorial Fund	2,000	2,000
<u>Research Projects, Fellowships, etc.:</u>				
Carnegie Corporation of New York:				
Biology:				
Yerkes Laboratories of Primate Biology	10,000	10,000
Geology:				
Tilley, C. E.	621	621
Physiology:				
Russell, G. Oscar	1,223	1,223
Natural Sciences:				
Fellowships	35,000	50,000	19,500	65,500
Terrestrial Magnetism:				
Telescope Image Converter	21,228	12,014	9,214
Total	\$134,591	\$383,537	\$155,329	\$362,799

EXHIBIT 3 CHANGES IN ENDOWMENT, GENERAL RESERVE, AND SPECIAL FUNDS
FOR THE YEAR ENDED JUNE 30, 1958

	Balance July 1, 1957	Realized Capital Gain, net	Income	Appropri- ations	Expendi- tures	Balance June 30, 1958
<u>Endowment Funds:</u>						
Endowment Fund	\$32,000,000	\$32,000,000
Capital Reserve Fund	19,611,525	\$47,112	19,658,637
<u>General Reserve Fund:</u>						
Unappropriated	3,500,986	\$1,571,177	(\$4,333,000)	739,163
Appropriated:						
Budget July 1, 1958 - June 30, 1959	2,233,000	2,233,000
Building Program	700,000	\$3,500	696,500
<u>Special Funds:</u>						
Colburn Fund	103,311	103,311
Harkavy Fund	5,051	5,051
Teeple Fund	10,888	10,888
van Gelder Fund	1,278	1,278
Bickel Fund	12,603	591	13,194
George E. Hale Relief Fund .	4,690	183	4,873
Harkavy Fund - Income	1,542	309	1,851
Harriet H. Mayor Relief Fund	8,100	800	7,300
Special Purpose Funds	76,423	2,109	3,620	82,152
Woloff Fund	21,466	1,006	22,472
Total	<u>\$55,357,863</u>	<u>\$47,112</u>	<u>\$1,573,286</u>	<u>(\$1,394,291)</u>	<u>\$4,300</u>	<u>\$55,579,670</u>

EXHIBIT 4

CHANGES IN BUILDINGS, LAND, AND EQUIPMENT FUND FOR THE YEAR ENDED JUNE 30, 1958

Classification of June 30, 1958 Balance							
	Balance July 1, 1957	Expendi- tures (Note)	Deduc- tions	Balance June 30, 1958	Buildings and Land	Library	Equipment
Departments of Research:							
Department of Plant Biology Stanford, California	\$172,703	\$12,192	\$66	\$184,829	\$75,520	\$29,315	\$79,994
Department of Genetics Long Island, New York	1,179,585	22,042	2,750	1,198,877	1,000,380	81,177	117,320
Geophysical Laboratory Washington, D. C.	496,027	22,067	518,094	170,384	53,114	294,596
Department of Archaeology Cambridge, Massachusetts	11,768	11,768
Mount Wilson Observatory Pasadena, California	1,700,111	13,985	1,714,096	275,828	85,118	1,353,150
Department of Terrestrial Magnetism Washington, D. C.	854,282	31,414	885,696	401,418	50,651	433,627
Department of Embryology Baltimore, Maryland	104,284	11,614	115,898	10,445	105,453
Total Departments of Research	\$4,518,760	\$113,314	\$14,584	\$4,617,490	\$1,923,530	\$309,820	\$2,384,140
Office of Administration							
Washington, D. C.	856,781	63,588	709	919,660	873,529	46,131
Total	\$5,375,541	\$176,902	\$15,293	\$5,537,150	\$2,797,059	\$309,820	\$2,430,271
Note: Current Expenditures for Equipment:							
Restricted Grants		\$2,500					
Operating Funds		165,224					
Prior Years' Expenditures Capitalized		9,178					
Total		\$176,902					

SCHEDULE 1

SECURITIES, JUNE 30, 1958
AND INCOME RECEIVED DURING THE YEAR

			Per Cent of Total Investments		
	Book Value	Approximate Market Value	Book Value	Approximate Market Value	Income Received
<u>Bonds:</u>					
United States Government	\$4,364,056	\$4,384,773	7.80	5.90	\$223,352
Foreign and International Bank	2,161,548	2,184,187	3.86	2.94	90,847
Public Utility	10,226,607	10,212,333	18.29	13.73	309,331
Communication	3,979,838	3,934,487	7.12	5.29	145,457
Railroad	368,588	355,975	0.66	0.48	21,410
Railroad Equipment Trust	537,514	543,140	0.96	0.73	17,032
Industrial and Miscellaneous	16,705,136	16,782,844	29.87	22.57	519,046
Total Bonds	<u>\$38,343,287</u>	<u>\$38,397,739</u>	<u>68.56</u>	<u>51.64</u>	<u>\$1,326,475 (a)</u>
<u>Stocks:</u>					
Preferred	\$2,604,641	\$2,470,177	4.66	3.32	\$117,391
Common	14,977,447	33,483,974	26.78	45.04	1,161,709 (b)
Total Stocks	<u>\$17,582,088</u>	<u>\$35,954,151</u>	<u>31.44</u>	<u>48.36</u>	<u>\$1,279,100</u>
Total	<u>\$55,925,375</u>	<u>\$74,351,890</u>	<u>100.00</u>	<u>100.00</u>	<u>\$2,605,575</u>

(a) After deducting bond premium amortization of \$24,533.

(b) Includes \$5,586 representing market value of a stock dividend received.

SCHEDULE OF SECURITIES

Principal Amount	Description	Maturity	Book Value	Approximate Market Value
United States Government Bonds				
\$665,000	U. S. of America Ctf. of Ind. 4s	1958	\$662,902	\$666,870
1,105,000	U. S. of America Treasury 2 $\frac{1}{4}$ s	1962-59	1,107,822 *	1,097,403
1,500,000	U. S. of America Treasury 2 $\frac{1}{2}$ s	1958	1,498,090	1,509,375
300,000	U. S. of America Treasury 2 $\frac{1}{2}$ s	1961	295,242	301,125
800,000	U. S. of America Treasury 2 $\frac{3}{8}$ s	1961	800,000	810,000
<u>\$4,370,000</u>	<u>Total U. S. Government</u>		<u>\$4,364,056</u>	<u>\$4,384,773</u>
Foreign and International Bank Bonds				
\$250,000	Aluminum Company of Canada, Ltd., S. F. Deb. 3 $\frac{7}{8}$ s Guar.	1970	\$252,454 *	\$254,062
500,000	Aluminum Company of Canada, Ltd., S. F. Deb. 4 $\frac{1}{2}$ s	1980	510,741 *	530,000
150,000	Australia, Commonwealth of, 4 $\frac{1}{2}$ s	1971	147,750	149,625
150,000	Australia, Commonwealth of, 5s	1972	150,000	157,125
250,000	British Columbia Power Commission, S. F. Deb. Series "L" 4 $\frac{3}{8}$ s	1987	245,000	261,875
125,000	International Bank for Reconstruction and Development, 3s	1976	125,000	117,500
125,000	International Bank for Reconstruction and Development, 3 $\frac{3}{8}$ s	1975	123,125	118,750
250,000	International Bank for Reconstruction and Development, 4 $\frac{1}{2}$ s	1977	250,000	261,250
150,000	Noranda Mines Ltd., S. F. Deb. 4 $\frac{3}{8}$ s	1968	152,798 *	150,000
200,000	Shawinigan Water & Power Co., 1st Mtg. & Coll. Tr. S. F. Series "M" 3s	1971	204,680 *	184,000
<u>\$2,150,000</u>	<u>Total Foreign and International Bank</u>		<u>\$2,161,548</u>	<u>\$2,184,187</u>
Public Utility Bonds				
\$250,000	California Oregon Power Company, 1st Mtg. 3 $\frac{7}{8}$ s	1986	\$253,099 *	\$240,000
125,000	Columbia Gas System, Inc., Series "B" 3s	1975	127,304 *	111,250
250,000	Columbia Gas System, Inc., Series "F" 3 $\frac{7}{8}$ s	1981	245,937	248,437
237,000	Columbus & Southern Ohio Electric Co., 1st Mtg. 3 $\frac{1}{4}$ s	1970	244,836 *	222,780
300,000	Commonwealth Edison Company, 1st Mtg. Series "R" 3 $\frac{1}{2}$ s	1986	300,729 *	288,000
300,000	Consolidated Edison Company of New York, Inc., 1st & Ref. Mtg. Series "L" 3 $\frac{5}{8}$ s	1986	303,837 *	297,900
300,000	Consolidated Edison Company of New York, Inc., 1st & Ref. Mtg. Series "N" 5s	1987	302,291 *	333,375
300,000	Consolidated Natural Gas Co., Deb. 2 $\frac{3}{8}$ s	1968	300,416 *	285,750
150,000	Consumers Power Company, 1st Mtg. 4s	1986	151,417 *	153,000
350,000	Consumers Power Company, 1st Mtg. 4 $\frac{3}{8}$ s	1987	352,198 *	379,750
300,000	Florida Power Corporation, 1st Mtg. 3 $\frac{7}{8}$ s	1986	302,132 *	295,500
500,000	Illinois Power Company, 1st Mtg. 3 $\frac{3}{4}$ s	1986	497,937	490,000
200,000	Minnesota Power & Light Co., 1st Mtg. 3 $\frac{3}{8}$ s	1975	202,807 *	182,000
250,000	Niagara Mohawk Power Corporation, Gen. Mtg. 3 $\frac{5}{8}$ s	1986	253,196 *	236,250
400,000	Niagara Mohawk Power Corporation, Gen. Mtg. 4 $\frac{1}{8}$ s	1987	403,418 *	435,000
100,000	Ohio Power Co., 1st Mtg. 3 $\frac{1}{4}$ s	1968	101,500	100,500
200,000	Pacific Gas and Electric Company, 1st & Ref. Mtg. Series "X" 3 $\frac{1}{8}$ s	1984	201,517 *	178,000
300,000	Pacific Gas and Electric Company, 1st & Ref. Mtg. Series "Y" 3 $\frac{3}{8}$ s	1987	306,497 *	282,750
250,000	Pacific Gas and Electric Company, 1st & Ref. Mtg. Series "BB" 5s	1989	251,937 *	275,625
200,000	Panhandle Eastern Pipe Line Co., Serial Deb. 2 $\frac{3}{8}$ s	1961-62	200,663 *	196,020
87,000	Panhandle Eastern Pipe Line Co., S. F. Deb. 3 $\frac{1}{4}$ s	1973	87,936 *	80,910
207,000	Philadelphia Electric Power Co., 1st Mtg. 2 $\frac{5}{8}$ s Guar.	1975	209,952 *	173,880
50,000	Philadelphia Electric Co., 1st & Ref. Mtg. 2 $\frac{7}{8}$ s	1978	49,687	44,250
500,000	Philadelphia Electric Co., 1st & Ref. Mtg. 4 $\frac{5}{8}$ s	1987	500,000	535,000
250,000	Potomac Electric Power Company, Deb. 4 $\frac{3}{8}$ s	1982	256,277 *	263,125
200,000	Public Service Company of Indiana, 1st Mtg. Series "F" 3 $\frac{1}{8}$ s ...	1975	202,878 *	187,000
400,000	Public Service Company of Indiana, 1st Mtg. Series "L" 4 $\frac{7}{8}$ s ...	1987	400,000	445,200
500,000	Public Service Electric & Gas Co., 1st & Ref. Mtg. 4 $\frac{1}{8}$ s	1987	504,622 *	543,750
250,000	Southern California Edison Company, 1st & Ref. Mtg. Series "G" 3 $\frac{5}{8}$ s	1981	247,765	246,250

* After deduction for amortization of premiums on bonds purchased subsequent to January 1, 1940.

SCHEDULE OF SECURITIES—Continued

Principal Amount	Description	Maturity	Book Value	Approximate Market Value
Public Utility Bonds—Concluded				
\$250,000	Southern California Edison Company, 1st & Ref. Mtg. Series "H" 4½s	1982	\$251,797 *	\$260,000
200,000	Southern California Edison Company, 1st & Ref. Mtg. Series "J" 4¾s	1982	202,125 *	216,500
210,000	Tennessee Gas Transmission Co., 1st Mtg. Pipe Line 2¾s	1966	211,260 *	197,400
191,000	Tennessee Gas Transmission Co., 1st Mtg. Pipe Line 3s	1969	194,094 *	177,630
300,000	Tennessee Gas Transmission Co., 1st Mtg. Pipe Line 5½s	1977	300,000	315,000
500,000	Union Electric Company, 1st Mtg. 3¾s	1986	500,107 *	492,500
265,000	United Gas Corporation, 1st Mtg. & Coll. Tr. 2¾s	1967	265,000	242,051
235,000	Virginia Electric & Power Company, 1st & Ref. Mtg. Series "M" 4½s	1986	239,439 *	244,400
300,000	Washington Water Power Company, 1st Mtg. 4¾s	1987	300,000	316,500
<u>\$10,157,000</u>	<u>Total Public Utility</u>		<u>\$10,226,607</u>	<u>\$10,212,333</u>
Communication Bonds				
\$150,000	American Telephone & Telegraph Company, Deb. 2¾s	1975	\$151,406 *	\$136,687
350,000	American Telephone & Telegraph Company, Deb. 3¾s	1984	361,012 *	323,750
800,000	American Telephone & Telegraph Company, Deb. 3¾s	1990	820,862 *	804,000
60,000	American Telephone & Telegraph Company, Conv. Deb. 4½s	1973	60,000	82,050
500,000	American Telephone & Telegraph Company, Deb. 4¾s	1985	505,817 *	530,000
400,000	Illinois Bell Telephone Company, 1st Mtg. Series "E" 4½s	1988	405,323 *	420,000
200,000	Mountain States Telephone & Telegraph Co., Deb. 3½s	1978	201,050 *	186,000
100,000	New York Telephone Co., Ref. Mtg. Series "E" 3½s	1978	100,937 *	92,250
200,000	Pacific Telephone & Telegraph Co., Deb. 3¼s	1978	203,311 *	182,000
300,000	Pacific Telephone & Telegraph Co., Deb. 4¾s	1988	307,152 *	315,750
250,000	Southern Bell Telephone & Telegraph Company, Deb. 4s	1983	251,298 *	257,500
300,000	Southern Bell Telephone & Telegraph Company, Deb. 5s	1986	306,670 *	331,500
300,000	Southwestern Bell Telephone Company, Deb. 3½s	1983	305,000 *	273,000
<u>\$3,910,000</u>	<u>Total Communication</u>		<u>\$3,979,838</u>	<u>\$3,934,487</u>
Railroad Bonds				
\$100,000	Chesapeake & Ohio Railway Co., Gen. Mtg. 4½s	1992	\$99,464	\$109,000
267,000	Fort Worth & Denver Railway Company, 1st Mtg. 4¾s Guar.	1982	269,124 *	246,975
<u>\$367,000</u>	<u>Total Railroad</u>		<u>\$368,588</u>	<u>\$355,975</u>
Railroad Equipment Trust Bonds				
\$250,000	Chicago Burlington & Quincy Railroad Co., Eq. Tr. 2¼s Guar. ..	1959-63	\$242,950	\$246,575
100,000	Great Northern Railway Company, Eq. Tr. 2s Guar.	1960-61	98,539	98,780
150,000	Pennsylvania Railroad Co., Eq. Tr. Series "S" 2¾s Guar.	1958-62	146,359	147,900
50,000	Southern Pacific Co., Eq. Tr. Series "CC" 2½s Guar.	1959	49,666	49,885
<u>\$550,000</u>	<u>Total Railroad Equipment Trust</u>		<u>\$537,514</u>	<u>\$543,140</u>
Industrial and Miscellaneous Bonds				
\$200,000	Allied Chemical and Dye Corporation, Deb. 3½s	1978	\$198,000	\$198,750
100,000	Aluminum Company of America, S. F. Deb. 3s	1979	100,000	92,000
200,000	Aluminum Company of America, S. F. Deb. 3½s	1964	200,000	200,500
250,000	Aluminum Company of America, S. F. Deb. 4½s	1982	250,000	262,500
187,000	American Tobacco Co., Deb. 3s	1969	188,465 *	185,130
234,000	Bristol-Myers Co., Deb. 3s	1968	234,559 *	219,960
500,000	Burroughs Corporation, Conv. Sub. Deb. 4½s	1981	538,478 *	572,500
550,000	C. I. T. Financial Corporation, Deb. 4¾s	1970	536,937	599,500
400,000	Commercial Credit Company, Notes 3¾s	1976	408,836 *	390,000
400,000	Continental Oil Company, S. F. Deb. 3s	1984	404,397 *	368,000
500,000	Crown Zellerbach Corporation, Promissory Note 4½s	1981	500,000	500,000
150,000	Dow Chemical Company, Deb. 2.35s	1961	150,170 *	147,375

* After deduction for amortization of premiums on bonds purchased subsequent to January 1, 1940.

SCHEDULE OF SECURITIES—Continued

Principal Amount	Description	Maturity	Book Value	Approximate Market Value
<u>Industrial and Miscellaneous Bonds—Concluded</u>				
\$130,000	Dow Chemical Company, Conv. Sub. Deb. 3s	1982	\$131,597 *	\$161,200
400,000	Federal Farm Loan Consolidated, The Twelve Federal Land Banks, 4s	1962	421,250	416,000
500,000	Federal Farm Loan Consolidated, The Twelve Federal Land Banks, 4½s	1970	498,826	535,000
153,000	Food Machinery Corporation, S. F. Deb. 2½s	1962	152,309	149,940
500,000	Food Machinery and Chemical Corporation, S. F. Deb. 3.80s ...	1981	500,000	502,500
400,000	Four Corners Pipe Line Company, Sec. Note 5s	1982	400,000	441,800
300,000	General American Transportation Corporation, Conv. Sub. Deb. 4s	1981	334,549 *	347,625
500,000	General Electric Company, Deb. 3½s	1976	502,237 *	498,125
250,000	General Motors Acceptance Corporation, Deb. 3s	1960	250,000	253,750
200,000	General Motors Acceptance Corporation, Deb. 3½s	1972	204,091 *	197,250
180,000	General Motors Acceptance Corporation, Deb. 4s	1958	180,000	179,831
200,000	General Motors Acceptance Corporation, Deb. 5s	1977	195,000	221,000
500,000	General Motors Corporation, Deb. 3¼s	1979	502,000 *	480,000
150,000	General Portland Cement Company, Conv. Sub. Deb. 5s	1977	154,375 *	185,250
275,000	Goodrich (B. F.) Company, 1st Mtg. 2¼s	1965	275,434 *	266,750
500,000	Illinois State Toll Highway Commission, Rev. Bds. 3¼s	1995	500,000	393,750
300,000	Kaiser Aluminum & Chemical Corporation, 1st Mtg. 5½s	1987	300,000	322,800
236,000	Lorillard (P.) Co., Deb. 3s	1963	238,070 *	232,460
500,000	National Cash Register Company, Conv. Sub. Deb. 4½s	1981	551,016 *	642,500
295,000	National Dairy Products Corporation, Deb. 2¾s	1970	297,429 *	274,350
488,000	Phillips Petroleum Co., S. F. Deb. 2¾s	1964	490,445 *	483,120
125,000	Pittsburgh Plate Glass Company, S. F. Deb. 3s	1967	125,000	124,531
150,000	Quaker Oats Co., Deb. 2½s	1964	148,922	145,500
100,000	Riegel Paper Corporation, S. F. Deb. 3¾s	1981	100,000	93,000
250,000	Scovill Manufacturing Company, Deb. 4¾s	1982	246,250	260,000
300,000	Seagram (Joseph E.) & Sons, Incorporated, Deb. 2½s	1966	298,500	273,000
525,000	Sears Roebuck Acceptance Corporation, Sub. Deb. 4½s	1977	511,505	544,687
300,000	Service Pipe Line Company, S. F. Deb. 3.20s	1982	300,000	282,000
500,000	Shell Union Oil Corporation, Deb. 2½s	1971	502,521 *	455,000
300,000	Sinclair Oil Corporation, Conv. Sub. Deb. 4¾s	1986	317,306 *	339,000
300,000	Superior Oil Company, The (Calif.) Deb. 3¼s	1981	300,000	300,000
300,000	Swift & Co., Deb. 2½s	1972	300,976 *	264,000
500,000	Texas Corporation, Deb. 3s	1965	510,716 *	505,000
250,000	Tide Water Associated Oil Company, S. F. Deb. 3½s	1986	250,000	232,500
500,000	Tremarco Corporation, Promissory Note 5s	1958	500,000	557,700
346,000	Union Oil Company of California, Deb. 2¾s	1970	352,403 *	323,510
400,000	Westinghouse Electric Corporation, Deb. 2½s	1971	402,567 *	364,000
250,000	Whirlpool-Seeger Corporation, S. F. Deb. 3½s	1980	250,000	225,000
500,000	F. W. Woolworth Company, Promissory Note 5s	1982	500,000	573,200
<u>\$16,524,000</u>	<u>Total Industrial and Miscellaneous</u>		<u>\$16,705,136</u>	<u>\$16,782,844</u>
<u>\$38,028,000</u>	<u>Bonds - Funds Invested</u>		<u>\$38,343,287</u>	<u>\$38,397,739</u>

Number of Shares	Preferred Stocks		
1,500	Appalachian Power Co., 4½% Cum. Pref.	\$159,000	\$151,875
1,500	Bethlehem Steel Corporation, 7% Cum. Pref.	183,638	236,625
3,800	Carrier Corporation, 4½% Cum. Pref.	197,931	174,800
600	Cleveland Electric Illuminating Co., \$4.50 Cum. Pref.	68,112	61,950
1,900	Consolidated Edison Company of New York, Inc., \$5.00 Cum. Pref.	202,816	204,725
600	Corn Products Refining Co., 7% Cum. Pref.	110,335	100,800
1,000	El Paso Natural Gas Co., 4.10% Cum. 1st Pref.	111,442	86,000
1,800	General Motors Corporation, \$5.00 Cum. Pref.	187,937	173,250
800	National Distillers & Chemical Corporation, 4¼% Cum. Conv. Pref.	80,000	74,200

* After deduction for amortization of premiums on bonds purchased subsequent to January 1, 1940.

SCHEDULE OF SECURITIES—Continued

Number of Shares	Description	Book Value	Approximate Market Value
<u>Preferred Stocks—Concluded</u>			
2,000	Niagara Mohawk Power Corp., 3.60% Cum. Pref.	\$207,990	\$156,000
1,300	Ohio Power Co., 4½% Cum. Pref.	144,630	129,350
1,500	Pacific Telephone and Telegraph Co., 6% Cum. Pref.	235,221	206,250
673	Pillsbury Mills, Inc., \$4.00 Cum. Pref.	72,497	66,627
2,000	Reynolds (R. J.) Tobacco Co., 3.60% Cum. Pref.	199,684	168,000
3,100	United States Steel Corporation, 7% Cum. Pref.	443,408	479,725
<u>23,773</u>	<u>Total Preferred Stocks</u>	<u>\$2,604,641</u>	<u>\$2,470,177</u>
 <u>Common Stocks</u>			
22,500	Aluminium Limited	\$453,746	\$599,062
3,000	Aluminum Company of America	125,062	209,625
16,074	American Electric Power Company, Inc.	214,286	689,173
5,500	American Telephone & Telegraph Company	787,124	984,500
180	Applied Science Corporation of Princeton	2,410	1,395
6,000	Armco Steel Corporation	225,534	301,500
14,100	Armstrong Cork Company	231,517	382,462
10,000	Atchison, Topeka and Santa Fe Railway Company	166,256	218,750
16,000	Bethlehem Steel Corporation	297,909	668,000
4,000	Caterpillar Tractor Company	96,914	252,000
3,000	Central & Southwest Corporation	110,250	145,500
2,708	Chase Manhattan Bank of New York	81,137	140,816
60	Christiana Securities Co.	356,143	744,000
4,200	Consumers Power Co.	145,974	221,025
4,600	Continental Can Company, Inc.	106,780	231,725
24,400	Continental Oil Company of Delaware	239,599	1,274,900
2,500	Corning Glass Works	59,632	208,125
9,656	Dow Chemical Company (The)	449,667	532,287
3,800	du Pont (E. I.) de Nemours & Co.	155,091	703,000
8,797	Eastman Kodak Company	209,366	976,467
6,840	First National City Bank of New York	348,175	450,585
6,000	Florida Power & Light Company	148,864	409,500
4,500	Ford Motor Company	243,789	186,750
34,000	General Electric Company	701,179	2,040,000
4,000	General Foods Corporation	83,651	255,000
15,000	General Motors Corporation	277,258	594,375
5,000	Goodrich (B. F.) Company	146,619	301,250
6,202	Goodyear Tire & Rubber Company	401,927	509,339
1,800	Great Northern Paper Company	174,191	85,050
5,512	Gulf Oil Corp.	95,278	640,081
10,625	Gulf States Utilities Co.	223,782	483,437
2,500	Halliburton Oil Well Cementing Company	47,814	152,500
1,600	Hudson's Bay Oil & Gas Co.	18,326	31,800
5,000	Illinois Power Co.	97,697	165,625
5,000	Insurance Company of North America	106,477	538,750
5,637.5	International Business Machines Corporation	203,470	2,080,237
5,000	International Nickel Co. of Canada, Ltd.	185,533	396,875
3,182	International Paper Company	135,058	312,631
6,500	Island Creek Coal Company	284,938	240,500
3,000	Kennecott Copper Corporation	153,175	267,750
8,640	Kimberly-Clark Corporation	182,251	486,000
9,400	Lehigh Portland Cement Company	278,295	316,075
1,305	Mellon National Bank & Trust Company	67,162	160,515
5,000	Merck & Co., Inc.	93,798	281,875
9,450	Minneapolis-Honeywell Regulator Co.	142,221	855,225
7,000	Monsanto Chemical Company	144,041	220,500
2,700	Northwest Bancorporation	187,854	191,700
3,300	Ohio Edison Company	105,150	180,675

SCHEDULE OF SECURITIES—Concluded

Number of Shares	Description	Book Value	Approximate Market Value
<u>Common Stocks—Concluded</u>			
8,000	Panhandle Eastern Pipe Line Company.....	\$431,554	\$388,000
4,500	Parke, Davis & Co.	354,082	369,000
2,400	Phelps Dodge Corporation	71,058	117,600
8,400	Procter & Gamble Co.	177,227	515,550
15,000	Puget Sound Power and Light Company	367,936	466,875
7,500	Royal Dutch Petroleum Co.	291,633	336,562
4,000	Scott Paper Company	53,042	270,500
12,342	Shell Oil Company	413,016	944,163
12,375	Socony Mobil Oil Company, Inc.	300,464	637,312
5,800	Southern California Edison Company	208,276	324,800
11,250	Southern Railway Company	218,509	458,437
31,402	Standard Oil Company of New Jersey	306,458	1,731,035
13,868	Texas Company	266,524	989,828
5,600	Texas Utilities Company	154,501	278,600
6,000	Travelers Insurance Company	452,663	455,250
5,000	Union Pacific Railroad Company	114,547	146,875
8,000	United States Gypsum Company	144,966	634,000
18,100	United States Steel Corporation	413,722	1,181,025
12,000	Virginia Electric and Power Company	130,448	361,500
8,000	West Virginia Pulp and Paper Company	226,534	304,000
6,800	Weyerhaeuser Timber Company	87,917	254,150
<u>551,105.5</u>	<u>Total Common Stocks</u>	<u>\$14,977,447</u>	<u>\$33,483,974</u>
	Common and Preferred Stocks - Funds Invested	\$17,582,088	\$35,954,151
	Aggregate Investments (Bonds and Stocks)	\$55,925,375	\$74,351,890

SUMMARY OF SECURITY TRANSACTIONS JULY 1, 1957 TO JUNE 30, 1958

Cash awaiting investment — July 1, 1957 \$51,850

Sales and Redemptions

	Gain	Loss	Book Value
Bonds	\$186,339	\$6,945,830
Preferred Stocks	161,713	827,204
Common Stocks	\$388,250	710,970
Sale of Stock Rights	6,783
Mortgages	131
	<u>\$395,164</u>	<u>\$348,052</u>	<u>\$8,484,004</u>
Net Gain - Statement B	47,112	47,112
	<u>\$395,164</u>	<u>\$395,164</u>	
Total Sales and Redemptions			8,531,116
Income applied to amortization of bond premiums			24,533
Gift of common stock			1,368
Market value of stock dividend			5,586
Surplus cash transferred for investment			200,000
Total			\$8,814,453

Acquisitions

Bonds	\$7,072,062
Common Stocks	1,730,764
Total Acquisitions	8,802,826
Cash awaiting investment - June 30, 1958	\$11,627

SCHEDULE 2

BUDGET SUMMARY OF OPERATING FUNDS FOR THE YEAR ENDED JUNE 30, 1958

	Disposition of Total Appropriations					
	Unexpended Appropriations	Unexpended Appropriations				
		Total Appropriations	Allot- ments	Continuing Appropriations	Total Expendi- tures	Trans- ferred to General Contingent Fund
	Unexpended Appropriations July 1, 1957	Budget Approp- riation for the fiscal year ended June 30, 1958				Reserved for Liabilities and Com- mitments and General Contingent Fund
Departmental Research Operations:						
Department of Plant Biology	\$2,268	\$83,750	\$2,400	\$73,046	\$3,165
Department of Genetics	5,950	177,500	19,411	195,292	3,655
Dormitory	3,500	1,200	\$11,032	11,190	4,542
Geophysical Laboratory	29,220	231,850	32,250	2,500	273,412	22,408
Department of Archaeology	2,650	59,650	(918)	43,961
Mount Wilson Observatory	25,942	294,000	3,600	284,436	21,331
Department of Terrestrial Magnetism	18,150	332,550	38,473	350,624	24,433
Department of Embryology	10,596	149,595	8,410	151,430	13,081
Total Departmental Research Operations	\$98,276	\$1,330,095	\$103,626	\$13,532	\$1,383,391	\$92,615
Administration	8,118	362,050	22,433	373,279	15,904
General Operations	40,610	207,741	(162,309)	86,042
General Publications	61,485	15,000	8,288	31,812	52,961
Research Projects, fellowships, etc.	245,969	196,858	179,270	263,557
Hospitalization Plan	8,060	12,000	3,044	22,422	682
Pension Fund	117,397	73,500	4,748	88,154	107,491
Retirement Plan Contributions	12,688	149,500	11,959	155,599	18,548
Total	\$592,603	\$2,149,886	\$180,359	\$21,820	\$2,233,927	\$637,800
General Contingent Fund	110,513	287,900	(180,359)	(72,941)	290,995
Total	\$703,116	\$2,437,786	\$21,820	\$2,233,927	\$928,795

ABSTRACT OF MINUTES OF THE SIXTIETH MEETING OF THE BOARD OF TRUSTEES

The annual meeting of the Board of Trustees was held in Washington, D. C., on Friday, May 9, 1958. The Chairman, Mr. Gifford, presided.

The following Trustees were in attendance: James F. Bell, Robert Woods Bliss, Omar N. Bradley, Walter S. Gifford, Caryl P. Haskins, Barklie McKee Henry, Ernest O. Lawrence, Alfred L. Loomis, Robert A. Lovett, Margaret Carnegie Miller, Henry S. Morgan, Seeley G. Mudd, William I. Myers, Henning W. Prentis, Jr., Elihu Root, Jr., Henry R. Shepley, Charles P. Taft, and James N. White.

The minutes of the Fifty-ninth Meeting were approved.

By unanimous action, Crawford H. Greenewalt and Robert E. Wilson were re-elected as members of the Board of Trustees.

The report of the President was accepted.

The report of the Executive Committee was accepted.

To provide for operation of the Institution for the fiscal year beginning July 1, 1958, and upon recommendation of the Executive Committee, the sum of \$2,233,000 was appropriated from the General Reserve Fund.

Other appropriations from the General Reserve Fund were: The sum of \$100,000 for the acquisition of a 60-foot-diameter parabolic radio telescope and the sum of \$700,000 to provide laboratory facilities for the Department of Embryology.

Reports of the Finance Committee, the Retirement Committee, the Auditor, and the Auditing Committee were accepted.

Vannevar Bush was elected a member of the Board of Trustees.

The following officers of the Board were re-elected for a period of three years: Walter S. Gifford, Chairman; Barklie McKee Henry, Vice-Chairman; and Robert Woods Bliss, Secretary.

The following were re-elected for three-year terms: Henry S. Morgan, Henning W. Prentis, Jr., and Henry R. Shepley as members of the Executive Committee; Walter S. Gifford, Alfred L. Loomis, and Henry S. Morgan as members of the Finance Committee; Alfred L. Loomis, Keith S. McHugh, and Juan T. Trippe as members of the Auditing Committee; Barklie McKee Henry as a member of the Retirement Committee; and Robert Woods Bliss as a member of the Nominating Committee. The following were elected or re-elected for one-year terms: Barklie McKee Henry as Chairman of the Executive Committee; James N. White as Chairman of the Finance Committee; Keith S. McHugh as Chairman of the Auditing Committee; and Elihu Root, Jr., as Chairman of the Nominating Committee.

ARTICLES OF INCORPORATION

Public No. 260. An Act to incorporate the Carnegie Institution of Washington

Be it enacted by the Senate and House of Representatives of the United States of America in Congress assembled, That the persons following, being persons who are now trustees of the Carnegie Institution, namely, Alexander Agassiz, John S. Billings, John L. Cadwalader, Cleveland H. Dodge, William N. Frew, Lyman J. Gage, Daniel C. Gilman, John Hay, Henry L. Higginson, William Wirt Howe, Charles L. Hutchinson, Samuel P. Langley, William Lindsay, Seth Low, Wayne MacVeagh, Darius O. Mills, S. Weir Mitchell, William W. Morrow, Ethan A. Hitchcock, Elihu Root, John C. Spooner, Andrew D. White, Charles D. Walcott, Carroll D. Wright, their associates and successors, duly chosen, are hereby incorporated and declared to be a body corporate by the name of the Carnegie Institution of Washington and by that name shall be known and have perpetual succession, with the powers, limitations, and restrictions herein contained.

Sec. 2. That the objects of the corporation shall be to encourage, in the broadest and most liberal manner, investigation, research, and discovery, and the application of knowledge to the improvement of mankind; and in particular—

(a) To conduct, endow, and assist investigation in any department of science, literature, or art, and to this end to cooperate with governments, universities, colleges, technical schools, learned societies, and individuals.

(b) To appoint committees of experts to direct special lines of research.

(c) To publish and distribute documents.

(d) To conduct lectures, hold meetings, and acquire and maintain a library.

(e) To purchase such property, real or personal, and construct such building or buildings as may be necessary to carry on the work of the corporation.

(f) In general, to do and perform all things necessary to promote the objects of the institution, with full power, however, to the trustees hereinafter appointed and their successors from time to time to modify the conditions and regulations under which the work shall be carried on, so as to secure the application of the funds in the manner best adapted to the conditions of the time, provided that the objects of the corporation shall at all times be among the foregoing or kindred thereto.

Sec. 3. That the direction and management of the affairs of the corporation and the control and disposal of its property and funds shall be vested in a board of trustees, twenty-two in number, to be composed of the following individuals: Alexander Agassiz, John S. Billings, John L. Cadwalader, Cleveland H. Dodge, William N. Frew, Lyman J. Gage, Daniel C. Gilman, John Hay, Henry L. Higginson, William Wirt Howe, Charles L. Hutchinson, *Samuel P. Langley*, William Lindsay, Seth Low, Wayne MacVeagh, Darius O. Mills, S. Weir Mitchell, William W. Morrow, *Ethan A. Hitchcock*, Elihu Root, John C. Spooner, Andrew D. White, Charles D. Walcott, Carroll D. Wright, who shall constitute

the first board of trustees. The board of trustees shall have power from time to time to increase its membership to not more than twenty-seven members. Vacancies occasioned by death, resignation, or otherwise shall be filled by the remaining trustees in such manner as the by-laws shall prescribe; and the persons so elected shall thereupon become trustees and also members of the said corporation. The principal place of business of the said corporation shall be the city of Washington, in the District of Columbia.

Sec. 4. That such board of trustees shall be entitled to take, hold, and administer the securities, funds, and property so transferred by said Andrew Carnegie to the trustees of the Carnegie Institution and such other funds or property as may at any time be given, devised, or bequeathed to them, or to such corporation, for the purposes of the trust; and with full power from time to time to adopt a common seal, to appoint such officers, members of the board of trustees or otherwise, and such employees as may be deemed necessary in carrying on the business of the corporation, at such salaries or with such remuneration as they may deem proper; and with full power to adopt by-laws from time to time and such rules or regulations as may be necessary to secure the safe and convenient transaction of the business of the corporation; and with full power and discretion to deal with and expend the income of the corporation in such manner as in their judgment will best promote the objects herein set forth and in general to have and use all powers and authority necessary to promote such objects and carry out the purposes of the donor. The said trustees shall have further power from time to time to hold as investments the securities hereinabove referred to so transferred by Andrew Carnegie, and any property which has been or may be transferred to them or such corporation by Andrew Carnegie or by any other person, persons, or corporation, and to invest any sums or amounts from time to time in such securities and such form and manner as are permitted to trustees or to charitable or literary corporations for investment, according to the laws of the States of New York, Pennsylvania, or Massachusetts, or in such securities as are authorized for investment by the said deed of trust so executed by Andrew Carnegie, or by any deed of gift or last will and testament to be hereafter made or executed.

Sec. 5. That the said corporation may take and hold any additional donations, grants, devises, or bequests which may be made in further support of the purposes of the said corporation, and may include in the expenses thereof the personal expenses which the trustees may incur in attending meetings or otherwise in carrying out the business of the trust, but the services of the trustees as such shall be gratuitous.

Sec. 6. That as soon as may be possible after the passage of this Act a meeting of the trustees hereinbefore named shall be called by Daniel C. Gilman, John S. Billings, Charles D. Walcott, S. Weir Mitchell, John Hay, Elihu Root, and Carroll D. Wright, or any four of them, at the city of Washington, in the District of Columbia, by notice served in person or by mail addressed to each trustee at his place of residence; and the said trustees, or a majority thereof, being assembled, shall organize and proceed to adopt by-laws, to elect officers and appoint committees, and generally to organize the said corporation; and said trustees herein named, on behalf of the corporation hereby incorporated, shall thereupon receive, take over, and enter into possession, custody, and management of all property, real or personal, of the corporation heretofore known as the Carnegie Institution, incorporated, as hereinbefore set forth under "An Act to establish a Code of Law for the District of Columbia, January fourth, nineteen hundred and two," and to all its rights, contracts,

claims, and property of any kind or nature; and the several officers of such corporation, or any other person having charge of any of the securities, funds, real or personal, books, or property thereof, shall, on demand, deliver the same to the said trustees appointed by this Act or to the persons appointed by them to receive the same; and the trustees of the existing corporation and the trustees herein named shall and may take such other steps as shall be necessary to carry out the purposes of this Act.

Sec. 7. That the rights of the creditors of the said existing corporation known as the Carnegie Institution shall not in any manner be impaired by the passage of this Act, or the transfer of the property hereinbefore mentioned, nor shall any liability or obligation for the payment of any sums due or to become due, or any claim or demand, in any manner or for any cause existing against the said existing corporation, be released or impaired; but such corporation hereby incorporated is declared to succeed to the obligations and liabilities and to be held liable to pay and discharge all of the debts, liabilities, and contracts of the said corporation so existing to the same effect as if such new corporation had itself incurred the obligation or liability to pay such debt or damages, and no such action or proceeding before any court or tribunal shall be deemed to have abated or been discontinued by reason of the passage of this Act.

Sec. 8. That Congress may from time to time alter, repeal, or modify this Act of incorporation, but no contract or individual right made or acquired shall thereby be divested or impaired.

Sec. 9. That this Act shall take effect immediately.

Approved, April 28, 1904

BY-LAWS OF THE INSTITUTION

Adopted December 13, 1904. Amended December 13, 1910, December 13, 1912, December 10, 1937, December 15, 1939, December 13, 1940, December 18, 1942, December 12, 1947, December 10, 1954, and October 24, 1957

ARTICLE I

The Trustees

1. The Board of Trustees shall consist of twenty-four members, with power to increase its membership to not more than twenty-seven members. The Trustees shall hold office continuously and not for a stated term.

2. In case any Trustee shall fail to attend three successive annual meetings of the Board he shall thereupon cease to be a Trustee.

3. No Trustee shall receive any compensation for his services as such.

4. All vacancies in the Board of Trustees shall be filled by the Trustees by ballot at an annual meeting, but no person shall be declared elected unless he receives the votes of two-thirds of the Trustees present.

ARTICLE II

Officers of the Board

1. The officers of the Board shall be a Chairman of the Board, a Vice-Chairman, and a Secretary, who shall be elected by the Trustees, from the members of the Board, by ballot to serve for a term of three years. All vacancies shall be filled by the Board for the unexpired term; provided, however, that the Executive Committee shall have power to fill a vacancy in the office of Secretary to serve until the next meeting of the Board of Trustees.

2. The Chairman shall preside at all meetings and shall have the usual powers of a presiding officer.

3. The Vice-Chairman, in the absence or disability of the Chairman, shall perform the duties of the Chairman.

4. The Secretary shall issue notices of meetings of the Board, record its transactions, and conduct that part of the correspondence relating to the Board and to his duties.

ARTICLE III

Executive Administration

The President

1. There shall be a President who shall be elected by ballot by, and hold office during the pleasure of, the Board, who shall be the chief executive officer of the Institution. The President, subject to the control of the Board and the Executive Committee, shall have

general charge of all matters of administration and supervision of all arrangements for research and other work undertaken by the Institution or with its funds. He shall prepare and submit to the Board of Trustees and to the Executive Committee plans and suggestions for the work of the Institution, shall conduct its general correspondence and the correspondence with applicants for grants and with the special advisers of the Committee, and shall present his recommendations in each case to the Executive Committee for decision. All proposals and requests for grants shall be referred to the President for consideration and report. He shall have power to remove, appoint, and, within the scope of funds made available by the Trustees, provide for compensation of subordinate employees and to fix the compensation of such employees within the limits of a maximum rate of compensation to be established from time to time by the Executive Committee. He shall be *ex officio* a member of the Executive Committee.

2. He shall be the legal custodian of the seal and of all property of the Institution whose custody is not otherwise provided for. He shall sign and execute on behalf of the corporation all contracts and instruments necessary in authorized administrative and research matters and affix the corporate seal thereto when necessary, and may delegate the performance of such acts and other administrative duties in his absence to the Executive Officer. He may execute all other contracts, deeds, and instruments on behalf of the corporation and affix the seal thereto when expressly authorized by the Board of Trustees or Executive Committee. He may, within the limits of his own authorization, delegate to the Executive Officer authority to act as custodian of and affix the corporate seal. He shall be responsible for the expenditure and disbursement of all funds of the Institution in accordance with the directions of the Board and of the Executive Committee, and shall keep accurate accounts of all receipts and disbursement. Following approval by the Executive Committee he shall transmit to the Board of Trustees before its annual meeting a written report of the operations and business of the Institution for the preceding fiscal year with his recommendations for work and appropriations for the succeeding fiscal year.

3. He shall attend all meetings of the Board of Trustees.

4. There shall be an officer designated Executive Officer who shall be appointed by and hold office at the pleasure of the President, subject to the approval of the Executive Committee. His duties shall be to assist and act for the President as the latter may duly authorize and direct.

5. The President shall retire from office at the end of the calendar year in which he becomes sixty-five years of age.

ARTICLE IV

Meetings

1. The annual meeting of the Board of Trustees shall be held in the City of Washington, in the District of Columbia, on the second Friday of May in each year, unless the date and place of meeting are otherwise set by order of the Executive Committee.

2. Special meetings of the Board may be called by the Executive Committee by notice served personally upon, or mailed to the usual address of, each Trustee twenty days prior to the meeting.

3. Special meetings shall, moreover, be called in the same manner by the Chairman upon the written request of seven members of the Board.

ARTICLE V

Committees

1. There shall be the following standing Committees, *viz.* an Executive Committee, a Finance Committee, an Auditing Committee, a Nominating Committee, and a Retirement Committee.

2. All vacancies occurring in the Executive Committee, the Finance Committee, the Auditing Committee, the Nominating Committee, and the Retirement Committee shall be filled by the Trustees at the next regular meeting. In case of vacancy in the Finance Committee, the Auditing Committee, the Nominating Committee, or the Retirement Committee, upon request of the remaining members of such committee, the Executive Committee may fill such vacancy by appointment until the next meeting of the Board of Trustees.

3. The terms of all officers and of all members of committees, as provided for herein, shall continue until their successors are elected or appointed.

Executive Committee

4. The Executive Committee shall consist of the Chairman, Vice-Chairman, and Secretary of the Board of Trustees and the President of the Institution *ex officio* and, in addition, five trustees to be elected by the Board by ballot for a term of three years, who shall be eligible for re-election. Any member elected to fill a vacancy shall serve for the remainder of his predecessor's term.

5. The Executive Committee shall, when the Board is not in session and has not given specific directions, have general control of the administration of the affairs of the corporation and general supervision of all arrangements for administration, research, and other matters undertaken or promoted by the Institution. It shall also submit to the Board of Trustees a printed or typewritten report of each of its meetings, and at the annual meeting shall submit to the Board a report for publication.

6. The Executive Committee shall have power to authorize the purchase, sale, exchange, or transfer of real estate.

Finance Committee

7. The Finance Committee shall consist of not less than five and not more than six members to be elected by the Board of Trustees by ballot for a term of three years, who shall be eligible for re-election.

8. The Finance Committee shall have custody of the securities of the corporation and general charge of its investments and invested funds, including its investments and invested funds as trustee of any retirement plan for the Institution's staff members and employees, and shall care for and dispose of the same subject to the directions of the Board of Trustees. It shall have power to authorize the purchase, sale, exchange, or transfer of securities and to delegate this power. It shall consider and recommend to the Board from time to time such measures as in its opinion will promote the financial interests of the Institution and of the trust fund under any retirement plan for the Institution's staff members and employees, and shall make a report at each meeting of the Board.

Auditing Committee

9. The Auditing Committee shall consist of three members to be elected by the Board of Trustees by ballot for a term of three years.

10. Before each annual meeting of the Board of Trustees, the Auditing Committee shall cause the accounts of the Institution for the preceding fiscal year to be audited by public accountants. The accountants shall report to the Committee, and the Committee shall present said report at the ensuing annual meeting of the Board with such recommendations as the Committee may deem appropriate.

Nominating Committee

11. The Nominating Committee shall consist of the Chairman of the Board of Trustees *ex officio* and, in addition, three trustees to be elected by the Board by ballot for a term of three years, who shall not be eligible for re-election until after the lapse of one year. Any member elected to fill a vacancy shall serve for the remainder of his predecessor's term, provided that of the Nominating Committee first elected after adoption of this By-Law one member shall serve for one year, one member shall serve for two years, and one member shall serve for three years, the Committee to determine the respective terms by lot.

12. Sixty days prior to an annual meeting of the Board the Nominating Committee shall notify the Trustees by mail of the vacancies to be filled in membership of the Board. Each Trustee may submit nominations for such vacancies. Nominations so submitted shall be considered by the Nominating Committee, and ten days prior to the annual meeting the Nominating Committee shall submit to members of the Board by mail a list of the persons so nominated, with its recommendations for filling existing vacancies on the Board and its Standing Committees. No other nominations shall be received by the Board at the annual meeting except with the unanimous consent of the Trustees present.

Retirement Committee

13. The Retirement Committee shall consist of three members to be elected by the Board of Trustees by ballot for a term of three years, who shall be eligible for re-election. Any member elected to fill a vacancy shall serve for the remainder of his predecessor's term, provided that of the Retirement Committee first elected after adoption of this By-Law one member shall serve for one year, one member shall serve for two years, and one member shall serve for three years, the Committee to determine the respective terms by lot.

14. The Retirement Committee shall, subject to the directions of the Board of Trustees, be responsible for the maintenance of a retirement plan for staff members and employees of the Institution and act for the Institution in its capacity as trustee under any such plan, except that any matter relating to investments under any such plan shall be the responsibility of the Finance Committee subject to the directions of the Board of Trustees. The Committee shall submit a report to the Board at the annual meeting of the Board.

ARTICLE VI

Financial Administration

1. No expenditure shall be authorized or made except in pursuance of a previous appropriation by the Board of Trustees, or as provided in Article V, paragraph 8, hereof.

2. The fiscal year of the Institution shall commence on the first day of July in each year.

3. The Executive Committee shall submit to the annual meeting of the Board a full statement of the finances and work of the Institution for the preceding fiscal year and a detailed estimate of the expenditures of the succeeding calendar year.

4. The Board of Trustees, at the annual meeting in each year, shall make general appropriations for the ensuing calendar year; but nothing contained herein shall prevent the Board of Trustees from making special appropriations at any meeting.

5. The Executive Committee shall have general charge and control of all appropriations made by the Board. Following the annual meeting each year, the Executive Committee may make allotment of funds for the period from January 1 to termination of the fiscal year on June 30. It may also make allotment of funds for the period from July 1 to December 31 in advance of July 1. The Committee shall, however, have full authority for allotment of available funds to meet necessary expenditures by other methods, if desirable, and transfer of balances to meet special needs. It shall make provision for outstanding obligations and for reversion of unexpended balances at termination of the fiscal year.

6. The securities of the Institution and evidences of property, and funds invested and to be invested, shall be deposited in such safe depository or in the custody of such trust company and under such safeguards as the Finance Committee shall designate, subject to directions of the Board of Trustees. Income of the Institution available for expenditure shall be deposited in such banks or depositories as may from time to time be designated by the Executive Committee.

7. Any trust company entrusted with the custody of securities by the Finance Committee may, by resolution of the Board of Trustees, be made Fiscal Agent of the Institution, upon an agreed compensation, for the transaction of the business coming within the authority of the Finance Committee.

ARTICLE VII

Amendment of By-Laws

1. These by-laws may be amended at any annual or special meeting of the Board of Trustees by a two-thirds vote of the members present, provided written notice of the proposed amendment shall have been served personally upon, or mailed to the usual address of, each member of the Board twenty days prior to the meeting.

INDEX OF NAMES

- Abell, George O., 21, 55, 67, 79
 publication, 85
- Abelson, Philip H., viii, 254, 255, 259
 publications, 163, 164, 257, 258, 259, 457
 report of Director of Geophysical Laboratory,
 167–260
 studies, 32, 214
- Adams, Walter S., 58
- Adams, William E., 371
 publication, 369
 studies, 40, 310, 320–321
- Afzelius, B., 313
- Agassiz, Alexander, vii, 479
- Ahrne, I., publication, 304, 305
- Aldrich, L. Thomas, viii, 165
 publication, 256, 259
 studies, 27, 176–181, 104–117
- Allen, M. B., 281
- Alter, Dinsmore, 79
- Anderson, Carl E., 122, 165
 publication, 163, 164
- Andrade, M. J., 442
- Appleman, D. E., 251
- Arnold, R. G., 253, 260
 studies, 30, 169, 218–222, 225
- Arnold, William A., xi, 35
- Arp, Halton C., viii, 84
 publication, 85
 studies, 15–16, 17, 51, 52, 67, 68–69, 69–70, 71,
 74, 78
- Arrhenius, Gustaf O., 254
- Ault, Wayne U., 254
- Baade, Walter, viii, 44, 49, 83–84
 publication, 85
 studies, 15, 16–17, 18, 19, 51, 52, 54–55, 66–67,
 70, 71, 73, 76
- Babcock, Harold D., 80
 publications, 85
 studies, 20, 55, 57
- Babcock, Horace W., viii, 45, 49, 84
 publications, 85
 studies, 19–20, 55, 62
- Bacher, Robert S., 49
- Baker, H. G., publication, 304
- Balbinder, Elias, 430
 studies, 390–406
- Balbinder, Evelyn, 430
 studies, 386–389
- Baldwin, George J., vii
- Balsley, J. R., publication, 163, 164
- Banič, Stanko, 377, 430
 studies, 376–377, 390–406
- Bappu, M. K. Vainu, 20, 55
- Barbour, Thomas, vii
- Barloutaud, R., publication, 163
- Barnes, H. L., 260
 publication, 258, 259
 studies, 30, 169, 234–240
- Bartelmez, George W., 371
 publications, 369, 371, 457
 studies, 318, 319, 351, 364–365, 367
- Barton, Paul B., Jr., 254, 259
 studies, 227–229
- Bassham, J. A., 283
- Bauer, Ailene J., x
- Baum, William A., viii, 84, 89
 publications, 85–86, 163
 studies, 16, 17, 18–19, 52, 54, 59, 68, 72–73, 74
- Bautzmann, H., 369
- Beard, Robert G., 368, 371
 studies, 336–337
- Becker, W., 68
- Bell, James F., v, vi, 461, 477
- Bellairs, Ruth, 325
- Benitez, A., publication, 304, 305
- Bergeron, John, 406
- Berkebile, Wilma J., 85
- Berlin, Heinrich, 451
- Bert, Grace R., 390, 431
- Biesecker, Earle B., x
- Billings, John S., vii, 479, 480
- Bishop, David W., ix, 310, 368, 369, 371
 publications, 369, 370
 studies, 38, 311–313, 338, 340
- Blaauw, Adriaan, 15, 51, 73
- Bliss, Robert Woods, v, vi, 461, 477
- Bluntschli Collection, 40, 310, 320, 321
- Boas, Franz, 435
- Boise, James W., x
- Bolton, Ellis T., viii, 165
 publications, 142, 163, 164, 457
 studies, 127–162
- Bonsack, Walter K., 85
 publications, 86
 studies, 60, 62
- Borgman, Jan, 79
- Böving, Bent G., ix, 368, 369, 371
 publications, 370
 studies, 313–317
- Bowen, Ira S., viii, 84
 publications, 86
 report of Director of Mount Wilson and Palo-
 mar Observatories, 49–88
- Bowen, N. L., 190, 207, 212
 publication, 256
- Boyd, Francis R., Jr., viii, 254, 255, 259
 studies, 29, 169, 170–174, 212, 249, 250

- Boyden, E. A., publication, 370
 Brachet, Jean, 408
 Bradford, Lindsay, v, 461
 Bradley, Omar N., v, 477
 Brainerd, G. W., studies, 24, 444, 451
 Brandtner, Friedrich, 255
 Britten, Roy J., viii, 165
 publications, 163, 164, 457
 studies, 127-162
 Broecker, W. S., 255
 Broggi, J., 107
 Brookings, Robert S., vii
 Brown, Jeannette S., 265
 studies, 281, 286-287
 Brown, Paul K., 357
 Buchanan, Jennie S., 430
 studies, 406-412
 Buddington, A. F., publication, 163, 164
 Burbidge, E. Margaret, publications, 86
 studies, 17, 21, 53, 67, 69
 Burbidge, Geoffrey R., publications, 86
 studies, 17, 53
 Burd, Sylvia, 84
 Burgh, Robert, 454
 Burgi, Elizabeth, 380, 381, 382, 383
 Burke, Bernard F., viii, 165
 publications, 163
 studies, 97-98, 101-104
 Burns, L. M., publication, 370
 Burns, Robert K., ix, 368, 371
 publication, 370
 studies, 349-350
 Bush, Vannevar, v, vii, 45, 444, 446, 477
 Buynitzky, S. J., 122, 166
- Cadwalader, John L., vii, 479
 California Institute of Technology, 14, 31, 49, 56, 243
 Callaway, Samuel, x
 Campbell, Allan M., 377, 430
 publications, 430
 studies, 37, 377, 386-389
 Campbell, William W., vii
 Cannon, W. Dilworth, Jr., 390, 431
 Cantolino, Salvatore J., 336, 371
 Carey, M. L., publication, 370
 Carnegie, Andrew, 480
 Carnegie Collection of Human Embryos, 310, 311, 317, 318, 364, 367
 Carnegie Corporation, 44, 310, 320, 371
 Carty, John J., vii
 Caspari, E. W., 377, 431
 Catcheside, David G., xi, 45, 377, 430
 Chao, Anna, 390, 431
 Chao, Edward C. T., 253
 Chayes, Felix, viii, 254, 255, 259
 publications, 257, 259
 studies, 30, 169, 204-206, 207, 240-243
 Cian, L. C., publication, 370
- Clark, L. A., 260
 studies, 30, 169, 229-232, 233, 246, 247, 248
 Clark, Sydney P., Jr., viii, 254, 259
 studies, 169, 174-176
 Clausen, Jens C., 266
 publications, 304, 457
 studies, 272-278
 Cleary, William, 365, 371
 Clegg, J. A., 165
 Clements, Leo, 371
 Clowes, R. C., publications, 430
 Code, Arthur D., viii, 84
 publication, 86
 studies, 15, 19, 51, 54, 64, 65, 71-72, 73, 77, 78, 79, 83
 Coffeen, Mary F., 84
 Coffman, C. M., 345
 Cohen, Georges N. P., 165
 publication, 163
 studies, 33, 144, 152, 153
 Cold Spring Harbor Symposium on Quantitative Biology, 378
 Cole, Whitefoord R., vii
 Coleman, John R., 368, 371
 Connelley, Mary, 67
Contributions to American Anthropology and History, 454
Contributions to Embryology, 310, 317, 319, 364, 367, 457
 Cook, A. F., 79
 Corliss, L. M., 31, 243, 254
 Corner, George W., 45, 314, 315, 368
 publication, 370, 457
 Corner, George W., Jr., 365
 Cowie, Dean B., viii, 165
 publications, 163, 164, 457
 studies, 127-162
 Cragg, Thomas A., 84
 publications, 86, 87
 studies, 56
 Creaser, E. H., 139
 Crick, F. H. C., 377
 Crowder, Roy E., 311, 371
 publication, 370, 457
 Crowell, P. S., publication, 370
 Csapo, Arpad, 314, 315
 Cuffey, J., 68
Current Reports, 447, 455
 Curtis, Howard, 406
- Datta, Mihir Kumar, 430
 Davies, R. D., 165
 Davis, Doreen, 318
 Davis, Gordon L., viii, 259
 publications, 163, 164, 256, 259
 studies, 27, 111-117, 169, 176-181
 Davis, H. J., publication, 370
 De, D. H., publication, 430
 Decker, John P., 266

- DeHaan, Robert L., ix, 368, 369, 371
 publication, 370
 studies, 322-326
- Dekaban, Anatole S., 318
- DeLanney, Louis E., 371
 publication, 370
 studies, 40, 310, 345, 346, 356
- Delano, Frederic A., vii
- Demerec, Milislav, ix, 430
 publications, 430
 report of Director of Department of Genetics,
 373-431
 studies, 36-37, 376, 390-406
- de Robichon-Szulmajster, H., studies, 33, 144, 165
- Deuchar, Elizabeth, 369
- Deutsch, Armin J., viii, 83, 84
 publications, 86
 studies, 18, 20, 53, 55, 61, 62-63
- Deutsch, Dorothy S., 84
- de Vries, Hessel, xi, 45, 253, 254, 255, 259
- DeVries, Pieter A., 371
 studies, 311, 317-318
- Dewhirst, David W., 84, 165
- Doak, J. B., 166
- Dodge, Cleveland H., vii, 479
- Dodge, William E., vii
- Donnay, Gabrielle, viii, 195, 254, 259
 publications, 256, 258, 259
 studies, 31, 169-170, 215, 217, 243-246, 248-
 249, 250, 252-253
- Donnay, J. D. H., publications, 256, 258, 259
 studies, 31, 215, 217, 243, 248-249, 250
- Dorsey, G. A., 435
- Dove, W. N., 166
- Drake, Charles W., 126
- Drane, Joseph P., 372
- Drosophila Information Service*, 378
- Duffey, L. M., publication, 370
- Duysens, L. N. M., 35, 264, 285, 297, 298
- Ebert, James D., ix, 310, 368, 369, 371
 publications, 370
 report of Director of Department of Embry-
 ology, 307-372
 studies, 39-40, 337-338, 340, 341, 342, 345-346,
 347, 356
- Ecklund, E. T., 166
- Elliott, Norman, 31, 243
- Elliott, Ruth F., 266
 studies, 272, 278-286, 303-304
- Elson, David, 138
- Emery, Kenneth O., 253, 254
- England, Joseph L., viii, 190, 259
 studies, 29, 169, 170-174
- Englesberg, E., 377, 431
- Epstein, Sam, 253, 254
- Erickson, W. C., 165
 publication, 163
 studies, 97, 101-104
- Ernst, W. G., 260
 studies, 30, 169, 199-204
- Espinas, Episanio E., 371
- Eugster, Hans P., viii, 254, 255, 259
 publication, 259
 studies, 29, 30, 169, 181-184, 191-194, 209-210,
 252
- Evans, H. T., 251
- Faul, H., 259
- Fehr, R. O., 453
- Fenner, Charles P., vii
- Ferguson, Homer L., vii
- Fine, William P., 265
- Firor, John W., viii, 165
 publications, 163
 studies, 97-98, 101-104
- Fisher, Agnes C., 430
- Flather, Edith, 66, 84
- Flexner, J. B., 129, 155, 165
- Flexner, Louis B., xi, 129, 155, 157, 165, 371
- Flexner, Simon, vii
- Flint, R. F., 255
- Forbes, W. Cameron, vii
- Forbush, Scott E., viii, 165
 publications, 163, 164, 457
 studies, 117-122
- Ford, W. Kent, Jr., viii, 92, 165
 publication, 85, 86, 163
- Forrestal, James, vii
- Fowler, William A., 17, 53
 publication, 86
- Frankel, S., publication, 163
- Franklin, K. L., publications, 163
- Fredericq, Pierre, 377, 390, 396, 430
- French, C. Stacy, ix, 265
 publications, 304
 report of Director of Department of Plant
 Biology, 261-305
 studies, 34, 278-287, 290-293, 300-301
- Frew, William N., vii, 479
- Friedman, Irving, 253, 255
- Fuscaldo, Kathryn E., 431
 studies, 406-412
- Gage, Lyman J., vii, 479
- Gardner, Ernest, publication, 370, 457
- Garen, Alan, 381
- Garrels, Robert M., 253, 254
- Gast, P. W., 116
- Gates, H. S., 74
- Gault, H. R., 259
- Gay, Helen, 430
 publications, 430
 studies, 375, 406-412
- Gettner, Mark, 406
- Giese, A. T., publication, 304, 305
- Giesecke, A. A., Jr., 118
- Gifford, Walter S., v, vi, 461, 477
- Gilbert, Cass, vii
- Gilbert, Perry W., publication, 370, 457

- Gillett, Frederick H., vii
Gilman, Daniel Coit, vii, 479, 480
Glanville, Edward V., 430
 studies, 390-406
Goedheer, Joop C., 265
 studies, 35, 264, 293, 295-303
Goffinet, John, 371
 studies, 346, 347
Goldman, Irving, 430
 publications, 430
 studies, 390-406
Goldsmith, Julian R., 253, 254
Goodgal, S. H., 377, 431
Gorodetsky, S., 165
Gots, J. S., 377, 431
Gottlieb, Hans B., 453
Graham, John W., viii, 165
 publications, 163, 164
 studies, 105
Gray, D. J., publication, 370, 457
Green, A. T., 254
Greenewalt, Crawford H., v, vi, 461, 477
Greenstein, Jesse L., viii, 49, 84
 publications, 86, 87
 studies, 18, 53, 59, 60, 61
Greenwald, G. S., 315
 publications, 370
Greig, Joseph W., viii, 213, 259
Grill, Richard D., 372
Grjebine, T., publication, 163, 164
Grodzinski, Z., 369
Gross, Julian D., 430
 studies, 390-406
Gryder, J. W., publication, 258, 259
Guggenheim, Paul, 371

Hack, Margherita, 83
 publication, 86
Hale, George Ellery, 20, 22, 55, 56
Hall, John S., 89
 publication, 85, 86, 163, 164
Halldal, Per, publications, 304
Ham, W. E., 181
Hansen, P. G., publication, 163, 164
Hanson, William E., 254
Harris, Daniel E., 85
Hart, R. W., 265, 290
 publication, 304, 305
Hartman, Carl G., 366
Hartman, Philip E., 390, 431
Hasek, Milan, 369
Hashimoto, Kazuo, 377, 430
 studies, 377, 390-406
Haskins, Caryl P., v, vi, x, 477
 publications, 457
 report of the President, 1-45
Hastings, Julius M., 31, 243
Hawkes, H. E., 253, 254
Haxo, Francis T., 265, 282, 283
Hay, John, vii, 479, 480

Hayden, R. J., 111
Hayes, W. C., 181
Heard, Osborne O., publication, 370, 457
Heilbrunn, L. V., 323
Helfer, H. Lawrence, 18, 59, 84
 studies, 97, 101-104
Hellman, Louis, 366
Hendricks, Sterling B., 303
Henry, Barklie McKee, v, vi, 461, 477
Herrick, Myron T., vii
Hershey, Alfred D., ix, 45, 430
 publications, 430
 studies, 36, 376, 379-386
Hertig, Arthur T., 371
Herzog, Emil R., 84
 publication, 86
 studies, 75
Heuser, Chester H., 371
 publication, 370, 457
 studies, 311, 317
Hewitt, Abram S., vii
Heydenburg, Norman P., viii, 165
 publications, 164
 studies, 122-127
Hickox, Joseph O., 56, 84
Hiesey, William M., ix, 266
 publications, 304, 457
 studies, 266-272, 272-278
Higginson, Henry L., vii, 479
Hiltner, W. A., 68, 78
Hitchcock, Ethan A., vii, 479
Hitchcock, Henry, vii
Hoering, T., 253
Holland, H., 253
Holmes, W. H., 435
Hoover, Herbert, vii
Hopson, C. A., 181
 publication, 256
Houck, T. E., publication, 86
 studies, 15, 51, 71-72, 77, 79
Howard, Robert F., 57, 84
Howarth, Sheila, 399
 publication, 430
Howe, William Wirt, vii, 479
Hoyer, B. H., 165
 publications, 142, 143, 164
Hoyle, Fred, viii, 84
 publications, 86, 87
 studies, 17, 53
Hubble, Edwin P., 73, 79, 81
 studies, 14-15, 16, 18, 51, 52, 54
Hughes, Vernon W., 126
Humason, Milton L., studies, 16, 18, 52, 54, 74,
 75
Hutchinson, Charles L., vii, 479

International Geophysical Year, 26, 27, 80, 95, 96,
 104, 105, 106, 107, 109, 110, 117, 119, 120,
 121, 162, 163
Ito, T., 250

- Jaffe, Lionel, 369
 James, H., 113
 Jenks, A. E., 436
 Jennings, Jesse D., studies, 23, 441
 Jensen, Einar, 111, 217
 Jessup, Walter A., vii
 Jewett, Frank B., vii
 Johnson, Clayton, 181
 Johnson, Harold, 68
 Johnson, Hugh M., 79–80
 Jones, H. W., Jr., publication, 370
 Jones, Henry H., 409, 430
 Jones, Thomas K., 80
Journal of Geophysical Research, 163
 Joy, Alfred H., publications, 87
 studies, 20, 55, 61

 Käfer, Etta, publication, 430
 Kanazir, D., 393
 Kane, R., 313
 Karossa, Judith, 430
 studies, 412–415
 Katsh, Seymour, 371
 publications, 370
 studies, 310, 338–340
 Kaudewitz, F., 377, 431
 Kaufmann, Berwind P., ix, 430
 publication, 430
 studies, 375, 406–412
 Kearns, Charles E., 59, 85
 Keck, David D., publication, 457
 Keenan, Philip C., publication, 86, 87
 Keith, M. L., 253, 254
 Kidder, Alfred V., 446
 studies, 23, 439, 440, 441, 443, 444, 451
 Kikkawa, H., 377, 431
 King, J. C., 377, 431
 Kingman, P., studies, 252–253
 Koch, Gebhard, 430
 studies, 379–386
 Kouvo, J. A. O., 29, 179
 Kozinsky, André, studies, 379–386
 Kracek, Frank C., 217, 234
 Kraft, Robert P., 63, 70
 Krauskopf, Konrad B., 253, 254
 Kuiper, G. P., 58, 82
 Kullerud, Gunnar, viii, 253, 254, 255, 259
 publications, 256, 258, 259
 studies, 30, 169, 215–218, 220, 222–229, 234,
 235, 246, 248–249
 Kupke, D. W., publication, 304, 305

 Lahr, Ernest L., 430
 publication, 430
 studies, 390–406
 Landolt, Elias, publication, 304
 Lange, Isabelle, publication, 164, 457
 Langley, Samuel P., vii, 479
 Larson, Carl L., publication, 142, 164

 Latimer, Paul H., 265
 publication, 304
 studies, 285, 293–295
 Laufer, Hans, 326, 368, 369, 371
 publication, 370
 studies, 41, 310, 333–336, 362–364
 La Velle, Arthur, 311, 371
 Lawrence, Ernest Orlando, v, vi, 43, 461, 477
 Lawrence, Lillian E., 449, 455
 Leahy, J. J., 165
 publication, 163, 164
 LeBouvier, George, publication, 142, 164
 Lederberg, J., publication, 164
 Lehmann, P., publication, 163, 164
 Lenhoff, H. M., 165
 Lennox, E. S., 392, 400
 Leopold, Luna B., 189
 Leupold, U., 377, 431
 Lévêque, A., publication, 163, 164
 Libby, Willard F., xi, 254
 studies, 31, 169, 184–189
 Lindbergh, Charles A., vii
 Lindsay, William, vii, 479
 Lindsley, Donald H., 105, 165
 Lippencott, E. R., 253
 Lipson, H., 112, 115, 177, 254
 Little, C. A., Jr., 166
 Livingston, William C., 80
 publication, 85, 87
 Lodge, Henry Cabot, vii
 Loeffler, J. E., publication, 304, 305
 Lombard, R. H., 222, 223, 225, 226
 Lomnitz, Cinna, 107, 110, 165
 Long, W. N., 365
 Loomis, Alfred L., v, vi, 461, 477
 Loomis, W. F., 157, 162
 Lovett, Robert A., v, vi, 477
 Low, Seth, vii, 479
 Lowen, A. Louise, 85
 Luria, S. E., 377, 382, 431
 Lutwak-Mann, Cecilia, 316, 369
 Luyten, Willem J., publication, 86, 87
 Lynch, Victoria H., publications, 304, 305
 studies, 300–301
 Lynds, R., 80

 McCleary, W. D., 345
 McClintock, Barbara, ix, 45, 430
 studies, 37–38, 375, 415–429
 McClure, F. T., 165
 publications, 163, 164
 MacDonald, Gordon J. F., 253, 254, 259
 McDonald, Margaret R., ix, 430
 studies, 38, 375–376, 412–415
 McHugh, Keith S., v, vi, 461, 477
 McIntyre, Jean W., 390, 431
 MacKenzie, W. S., 259
 publications, 257, 259
 McNamara, D. H., 81
 MacVeagh, Wayne, vii, 479

- Makinodan, Takashi, 344, 369
 Mandel, H. G., 165
 publication, 163, 164
 Mandell, Joseph D., 431
 studies, 379-386
 Markert, C. L., 369
 studies, 362-363
 Marshall, D. R., 165
 Marshall, J. M., publications, 370
 Marshall, Leona, 84
 Marton, L. L., 89
 Mason, David T., 266
 Mathis, John, 80
 Matthews, Mildred S., 85
 publication, 86, 87
 Matthews, Thomas A., 84
 studies, 70, 76
 Mayall, N. U., 18, 54, 65
 Melbourne, William G., 61, 69, 85
 Melechen, Norman E., 381, 382
 Mellon, Andrew W., vii
 Merriam, John Campbell, vii, 309, 437, 438, 439, 444
 Merrill, Paul W., xi, 84
 publications, 87, 457
 studies, 20, 55, 61, 62
 Merritt, C. A., 181
 Merwin, H. E., 197, 198, 222, 223, 225, 226, 259
Microbial Genetics Bulletin, 378
 Miller, Margaret Carnegie, v, vi, 461, 477
 Miller, Roswell, vii
 Miller, William C., 85
 Mills, Darius O., vii, 479
 Milner, Harold W., ix, 265
 studies, 266-270
 Milner, Max, 265
 Milton, Charles, 253, 254
 studies, 29, 169, 181-184
 Mineur, Henri, 15, 51, 73
 Minkowski, Rudolph L., viii, 44, 49, 84
 publications, 87
 studies, 19, 20, 54, 55, 65, 66, 72, 74-75, 76
 Mitchell, S. Weir, vii, 479, 480
 Miyake, Tadashi, 431
 studies, 390-406
 Moffett, Benjamin C., Jr., publication, 370, 457
 Montague, Andrew J., vii
 Moore, Marianne Jacobs, 350, 372
 Morey, G. W., 254
 publications, 256, 258, 259
 studies, 190-191
 Morgan, H. R., 15, 51, 73
 Morgan, Henry S., v, vi, 461, 477
 Morgan, Russell, 367
 Morgan, W. W., 81
 Morimoto, N., 255, 260
 studies, 30, 31, 169, 212-213, 246-248, 249-252
 Morley, Sylvanus G., studies, 22, 23, 436-437, 437-438, 440, 441, 442, 445
 Morris, Earl H., studies, 24, 440
 Morrow, William W., vii, 479
 Moser, Hermann, 377, 431
 publication, 430, 457
 Mouquin, Catharine, 165
 Mudd, Seeley G., v, vi, 461, 477
 Mulnard, Jacques, 310, 346, 371
 Münch, Guido, viii, 84
 publications, 87
 studies, 19, 20, 54, 55, 64, 65, 72
 Münch, Luis, 64, 81
 Myers, William I., v, vi, 461, 477
 Nathan, O., publication, 163, 164
 National Bureau of Standards, 89, 162, 259, 291
 National Geographic Society-Palomar Mountain Sky Survey, 21, 55, 67, 75
 Naval Observatory, U. S., 56, 59, 89, 91
 Neher, H. V., 119
 Newburn, Ray L., publication, 87
 Nichols, Richard F. F., x
 Nicholson, Seth B., publications, 87
 studies, 20, 21, 55, 56
 Nicolaysen, L. O., 165
 Nobs, Malcolm A., ix, 266
 studies, 266-272, 272-278
 Noller, C. R., 288
 Norton, Robert G., 68, 85
Notes on Middle American Archaeology and Ethnology, 455
 Noyes, A. A., 31, 243
 Odgers, G. J., 81
 Oke, J. Beverly, viii, 84
 Oksala, T., 377, 431
 Ondik, H. M., publication, 258, 259
 Oort, Jan Hendrick, xi, 84
 studies, 19, 54, 70, 72
 O'Rahilly, Ronan, publication, 370, 457
 Ormsbee, Richard A., publications, 142, 143, 164
 Orville, P. M., 260
 studies, 206-209
 Osborn, E. F., 198
 Osborn, William Church, vii
 Osterbrock, Donald E., viii, 59, 65, 84
 publications, 87
 studies, 17, 20, 52, 55, 66, 74-75
 Ozeki, Haruo, 395
 Padget, Dorcas H., publication, 370, 457
 Palacas, J. G., 259
 Parmelee, James, vii
 Parsons, Wm. Barclay, vii
 Paton, Stewart, vii
 Pawsey, J. L., 165
 Pederson-Krag, Gillian, 406, 431
 Pepper, George W., vii
 Pershing, John J., vii
 Pestell, Wilbur A., 266
 Pettijohn, Francis J., 112, 115, 177

- Pettit, Edison, 18, 54
Phage Information Service, 378
Pieper, G. F., 122, 165
publications, 164
Pogo, Alexander, 85
Pollard, E. C., 165
Pollock, Harry E. D., ix, xi, 25, 446, 449, 454, 455
publication, 455
report of Director of Department of Archaeology, 433-455
Popper, Daniel, 81-82
Pray, Thomas R., 266
Prentis, Henning W., Jr., v, vi, 461, 477
Pritchett, Henry S., vii
Proskouriakoff, Tatiana, ix, 449, 455
publications, 454, 455
studies, 448, 451
Pumpelly, Raphael, 435-436

Quidort, J., publication, 163, 164

Ramberg, Hans, xi
Ramsey, Elizabeth M., ix, 368, 369, 371
publication, 371
studies, 365-367
Rankin, G. A., 197, 198
Raper, Kenneth B., 325
Rawles, Mary E., ix, 369, 371
studies, 310, 319-320
Ray, Clayton E., publication, 455
Reed, George W., 253, 254
Rentschler, Gordon S., vii
Rever, Arthur G., 354, 365, 372
Reynolds, Samuel R. M., 366, 371
Richardson, Robert S., viii, 84
publication, 87
studies, 20, 55, 56, 58
Ricketson, O. G., 441
Ringwood, A. E., 253
Ritter, Daniel B., publications, 142, 143, 164
Rivers, W. H. R., 436
Robbins, C. R., 207, 259
Roberts, I. Z., 165
Roberts, Richard B., viii, 165
publications, 132, 164, 457
studies, 127-162
Robertson, D. R., 290
Rockefeller, David, vii
Roemer, Elizabeth, 59
Root, Elihu, vii, 479, 480
Root, Elihu, Jr., v, vi, 461, 477
Roper, J. A., 377, 431
Roques, Paul, 61
Rose, D. C., 118
Roseboom, E. H., Jr., 253, 260
studies, 30, 169, 222-227, 232-234
Rosenwald, Julius, vii
Rossman, I., 367
publication, 371
Roys, Ralph L., 446
publication, 454

Rubey, W. W., 253
Rubin, Meyer, 255
Rule, Bruce, 85
Russell, H. E., 166
Ruth, Royal F., ix, 310, 371
studies, 326-333, 350-356
Ryerson, Martin A., vii

Saa, P. German, 107, 165
Sahade, Jorge, 82
publication, 87
Sahama, Th. G., publication, 257, 259
Sams, Joan, 431
Sandage, Allan R., viii, 45, 81, 84
publications, 86, 87
studies, 15, 16, 17, 18, 21, 51, 52, 54, 67, 68, 69, 70, 72, 73-74
Sanders, William R., 451
publication, 455
Sanford, Roscoe F., publication, 86, 87
studies, 20, 55, 61
Schairer, J. Frank, viii, 255, 259
publications, 256, 259
studies, 30, 169, 197-199, 208, 210-213, 249, 250
Schalet, A., 431
Scherer, Paul A., x
Schmidt, Maarten, 84
studies, 17, 52, 64, 66, 68, 69, 77
Schmidt, R., 204
Schneiderman, Howard, 362
Schreyer, W. F., 260
studies, 30, 169, 195-199
Schulte, Charles J. A., III, 325, 371
Schwarzschild, Martin, 17, 53
Scott, Roger B., 311, 371
publication, 371
Scott, W. E., 166
publication, 164
Sengün, Atif, 407
Sensenig, E. Carl, 311, 371
publication, 371, 457
Settle, George W., 311, 364, 371
Seyfert, Carl, Jr., 56
Shaffer, B. M., 325
Shepard, Anna O., ix, 455
publication, 457
studies, 448, 449, 450, 451-454
Shepley, Henry R., v, vi, 461, 477
Shibata, K., publication, 305
Shook, Edwin M., ix, 455
publications, 455
studies, 23, 441, 449, 451
Shull, G. H., 36
Simonsen, Morten, 369
studies, 343, 344-345, 346
Simrell, Elizabeth Jane, 431
Singer, A. D., 260
Singer, Marcus, studies, 333-336
Sinton, William M., 82
Sletteback, Arne, 82

- Slizynska, H., 377, 431
Slizynski, B. M., 377, 431
Smith, A. Ledyard, ix, 455
 studies, 448
Smith, E. Ide, publication, 371, 457
Smith, Evelyn E. B., 45
Smith, Guinevere C., 378, 431
Smith, Harlan J., publication, 87
Smith, J. G., publication, 256
Smith, J. R., 189, 190, 260
 publication, 256
Smith, James H. C., ix, 265, 266
 publications, 304, 305
 studies, 34, 35, 263, 264, 281, 286, 287-295
Smith, M. B., 165
Smith, Robert E., ix, 455
 publication, 455
 studies, 448, 450-451, 452
Smith, Theobald, vii
Smith, Watson, 454
Snyder, Emmy M., 390, 431
Solecki, Ralph, 255
Sorokin, Constantine, publication, 305
Spangler, C., 259
Spoonier, John C., vii, 479
Staber, Elaine, 390, 431
Stebbins, Joel, 16, 18, 52, 54
Steinberg, Malcolm S., 310, 368, 369, 371
 publication, 371
 studies, 321-322, 325
Stephens, Cynthia, 70, 85
Steuart, Charles D., 371
 studies, 347-349
Stewart, D. B., 189, 190, 253, 259
Storey, William Benson, vii
Strain, Harold H., publication, 305
Stran, Herbert, 365
Strehler, Bernard L., publication, 305
Streisinger, George, ix, 377, 380, 431
Strömrgren, Bengt, 66
Strong, John, 82
Strong, Richard P., vii
Struve, Otto, 63, 82-83
 publication, 86, 87
Stryker, Lucile B., x
Sullivan, James F., x
Swanson, Oscar, 85
Swings, Pol, 59
 publication, 87
Swope, Henrietta, 71, 85

Taft, Charles P., v, vi, 461, 477
Taft, William H., vii
Tanner, Neal, 84
Tatell, Howard E., viii, 43-44, 165
 publications, 164
 studies, 97, 101-111
Teeple, J. E., 440, 442

Temmer, Georges M., viii, 165
 publications, 163, 164
 studies, 122-127
Thackeray, A. D., 15, 51, 72
Thatcher, L. L., 189, 259
Thayer, William S., vii
Thomas, C., 382
Thomas, J. O., 165
Thomas, René, 431
 studies, 379-386
Thompson, J. Eric S., ix, 449
 publications, 455
 studies, 440, 442, 445, 446-447, 448-449
Thompson, James B., 253, 254
Tift, Carol, 85
Tift, William G., 85
 publications, 87
 studies, 19, 54, 61, 73
Tilley, C. E., xi, 189, 205
Tilton, George R., viii, 254, 255, 259
 publications, 164, 256, 259
 studies, 27, 111-117, 169, 176-181
Tomizawa, Jun-ichi, 431
 studies, 379-386
Toole, E. H., 303
Trippe, Juan T., v, vi, 461, 477
Turnock, A. C., 260
 studies, 30, 169, 191-193, 209-210
Tuttle, O. F., 190, 207
 publication, 256
Tuve, Merle A., viii, 165
 publications, 164
 report of Committee on Image Tubes for Tele-
 scopes, 89-92
 report of Director of Department of Terrestrial
 Magnetism, 93-166
 studies, 97, 101-111

Vaillant, George C., studies, 23, 438
Vallentyne, J. R., 260
van de Kamp, Peter, 83
Van Deman, E. B., 436
van den Bergh, Sidney, 18, 69, 70, 83
Van Devender, Russell R., Jr., 85
Venkatesh, C. S., publication, 305
Vestine, E. H., publication, 164
Virgin, Hemming I., publication, 304, 305
Visconti, N., 381
Vogel, H. J., 377, 393, 431
von Wettstein, D., 377, 431

Wadsworth, James W., vii
Walcott, Charles D., vii, 479, 480
Walcott, Frederic C., vii
Walcott, Henry P., vii
Wald, George, 357, 361
Walker, Merle F., publication, 87
 studies, 17, 52, 83
Wallace, Bruce, 377, 431

- Wallerstein, George, 84
 publications, 86, 87-88
 studies, 18, 59, 60, 61, 68, 69
 Wasserburg, G. J., 111, 112, 115, 116, 117, 165, 177
 Waters, Aaron C., 105, 181
 Watson, Earnest C., 49
 Weaver, H., 165
 Weberg, Bert, 453
 Weed, Lewis H., vii
 Weidemann, Volker, 60, 84
 Welch, William H., vii
 Wells, Harry W., viii, 165
 publications, 164
 studies, 97, 99-101
 Welte, D., 259
 Wesselink, A. J., 15, 51
 Westergaard, Mogens, xi, 45, 377, 431
 Wetherill, George W., viii, 165
 publications, 164, 256, 259
 studies, 27, 104-117, 176-181
 Wharton, Lawrence R., Jr., 311, 371
 publication, 371
 White, Andrew D., vii, 479
 White, Edward D., vii
 White, Henry, vii
 White, James N., v, vi, 477
 White, John I., studies, 332-333
 Whitford, A. E., 16, 18, 52, 54
 Whittaker, E. J. W., 200
 Wickersham, George W., vii
 Wild, P., publication, 86, 88
 Wildey, Robert L., 58, 62, 85
 Willey, Gordon R., 448, 454
 Willier, B. H., 310, 368, 369
 Wilson, Albert G., publication, 87
 Wilson, Carole E., 431
 Wilson, Katherine, 411
 Wilson, Olin C., viii, 84
 publications, 87
 studies, 20, 53, 55, 64-65, 78
 Wilson, Ralph E., 20, 55
 Wilson, Robert E., v, vi, 461, 477
 Wilt, Fred H., 369, 371
 studies, 357-361
 Witkin, Evelyn M., xi, 377, 378, 392, 431
 Witschi, Emil, 311, 371
 Wones, D. R., 260
 studies, 30, 169, 193-195, 210, 252
 Woods, Philip S., 407
 Woodward, Robert Simpson, vii
 Woolley, Richard v. d., xi, 64, 84
 Wootton, Jennifer M., 266
 studies, 278
 Wright, Carroll D., vii, 479, 480
 Wytttenbach, Charles R., 371
 publication, 370, 371
 studies, 40, 340-343
 Yoder, Hatten S., Jr., viii, 45, 255, 259
 publications, 256, 257, 259
 studies, 30, 169, 173, 189-191, 192, 195-197,
 210-212, 222, 225, 226, 227, 228, 234, 235
 Yoshida, Yoko, 431
 Yura, Takashi, 399
 Zies, E. G., 259
 studies, 204-206
 Zwicky, Fritz, viii, 84
 publications, 86, 87
 studies, 19, 54, 60, 74, 75

